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Vol. XXXII, Part I 1949

STUDIES ON *SCLEROTINIA* AND *BOTRYTIS*

II. DE BARY'S DESCRIPTION AND SPECIMENS OF *PEZIZA FUCKELIANA*

By P. H. GREGORY
Agricultural Research Council Research Officer at Rothamsted Experimental Station, Harpenden, Herts

(With Plates I–III and 1 Text-figure)

Botrytis cinerea Fr. is a facultative parasite with a wide host range in temperate regions. The perfect stage was named *Peziza fuckeliana* by de Bary in 1866, and transferred to the new genus *Sclerotinia* by Fuckel in 1869. The connexion between *Botrytis cinerea* and *Sclerotinia fuckeliana* was generally accepted until the beginning of this century, when the adoption of pure-culture methods in plant pathology aroused doubts of the correctness of the conclusions de Bary drew from his observations. Isolates of *Botrytis cinerea* could be maintained *in vitro* for many years without producing apothecia from the sclerotia. Authorities such as Lind (1913) and Brierley (1931) even denied the existence of a *Sclerotinia* stage, Klebahn (1930) regarded the question as needing a new investigation, and the theory fell into disrepute.

However, apothecia were produced in pure cultures of *Botrytis* by several workers, including Seaver and Horne (1918), Seaver (1947), Godfrey (1919, 1923) and van Beyma Thoe Kingma (1927). The connexion was firmly established when Drayton (1937) with *B. convoluta*, and Groves and Drayton (1939) with *B. cinerea*, studied the conditions under which apothecia were formed in culture, and described how they could be produced at will. Their method involved spermatizing sclerotia of *Botrytis* isolates by microconidia from other compatible isolates, combined with suitable cold treatment. The failure of earlier workers to obtain the *Sclerotinia* stage from pure cultures of *Botrytis* is thus due directly to the purity of the technique used, which provided no mechanism for the transfer of microconidia to the receptive bodies of the same or other isolates. The methods of Drayton and Groves were subsequently used by Buddin (in Dennis & Wakefield, 1946) with a new species *Sclerotinia draytoni* from *Gladiolus*. The connexion has also been confirmed by me for *Sclerotinia narcissicola*, *S. polyblastis*, *S. sphaerosperma*, and an unnamed species of *Sclerotinia*, with apothecia produced out of doors from naturally infected plant material (Gregory, 1940, 1941).

The question arises whether all species of *Sclerotinia* arising from sclerotia of *Botrytis cinerea* belong to *Sclerotinia fuckeliana*. The problem has been complicated by uncertainty as to what fungus de Bary had, because his specimens and description have been overlooked hitherto. Saccardo cites 'De Bary, *Morph. Pilz.* p. 30' (1866) for the description, but in the passage

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referred to de Bary merely stated that '*P*(*eziza*) *Fuckeliana* n.sp.' was to be described in detail elsewhere. Search by me in the usual sources failed to reveal any recognizable description by de Bary of *Sclerotinia fuckeliana*. Eventually Dr G. R. Bisby noticed a figure in Zopf's *Die Pilze*, accompanied by a reference to an obscure paper of de Bary's which proved to contain the missing description and illustration of *Peziza fuckeliana*. Dr J. Ramsbottom then drew my attention to de Bary's collection of microscope slides in the Department of Botany, British Museum (Natural History). This contains a slide with sections of apothecia labelled '*Peziza fuckeliana*, 22/10/64', which must constitute the type specimen. The following account summarizes the information on this fungus available from various sources, and should help to clarify the position of apothecia now obtained in culture.

1797. *C. H. Persoon. Tentamen dispositionis methodicae fungorum.* Lipsiae.

On p. 40 the fungus is referred to as '*Botrytis. B. cinerascens, sparsum, ramosum ex albido cinerascens. tab.* 3. *fig.* 9. (*I in fol. putrid. brss. olerac.*'), but p. 47, fig. 9, refers to this fungus as '*Botrytis cinerea*...' and this is apparently the first use of the name.

1801. *C. H. Persoon. Synopsis methodica fungorum.*

P. 690 gives the following description:

'*Botry. cinerea: congesta late effusa ramosa cinerea.* Disp. fung. p. 46, t. 3. fg.

'Hab. in *Cucurbitus* putrescentibus, et ad caules *Brassicae oleraceae*. Sub lente illustrata formam distinctam sistit, ceteroquin *Bysso* similis.'

1832. *E. Fries. Systema Mycologicum*, III, ii.

On pp. 396–7 under *Botrytis* the fungus is described as follows:

'*B. cinerea*, floccis fertilibus gregariis sub simplicibus cinereis, mox *strangulatis*, sporidiis hinc inde accumulatis globosis albidioribus.

'*B. cinerea. Pers. Syn. p.* 690 *disp. meth. t.* 3. *f.* 10. [? fig. 9] haud bona! *Alb. et Schwein. Consp. p.* 363. *Schum. Saell.* 2. *p.* 238. Link. *Spec.* l. *p.* 63.

'A praecedente [*B. grisea*] longe removeri non debet. Flocci steriles in hac saepe etiam evanescunt, sed fertiles in densam silvulam, lineam altam, stipantur, jam nudo oculo distinguuntur farinosi; omnino simplices aut uno alterove ramo simplici aucti. Sub. micr. comp. flocci adulti semper strangulati, articulis alternis constrictis, alternis compressis, ut quasi *Alternaria* sui generis. Sporidia glomerata, globosa, *alba*, per aetatem cinerea. Duae praecipue sunt hujus speciei formae habitu valde recedentes, sed characteribus haud separandae; altera ad caules exsiccatos nudo oculo magis fructiculosa, rigida, cinerea; altera ad caules putredine fere dissolutos, haec mollis, mucidinea, fuligineo-cinerea. *Ad caules plantarum emortuos vulgatissima omni anni tempore* (v.v.).'

This description is the nomenclatural type of *Botrytis cinerea* under the Rules of Botanical Nomenclature, until a type or lectotype specimen may be found or designated. It is of interest to note that even as early as the time of Fries it was recognized as being a variable species.

Sclerotinia fuckeliana

1866. *A. de Bary* in W. Hoffmeister's *Handbuch der Physiol. Botanik*, 2. Bd. 1866. *Morphologie und Physiologie der Pilze, Flechten und Myxomyceten*, Leipzig, 8. xii u. 316 S.

The first reference to the fungus appears on p. 30 in a list of fungi which arise from sclerotia: '*P. Fuckeliana* n.sp.[1] (*Scl. echinatum* Fuck.).' The footnote reads:

'[1] Mit obigem Namen bezeichne ich die anderweitig ausführlich zu beschreibende der *P. Candolleana* nahe verwandte Peziza, die auf Rebenblättern kleine schwarze Sclerotien (*Scl. echinat.* F) bildet.'

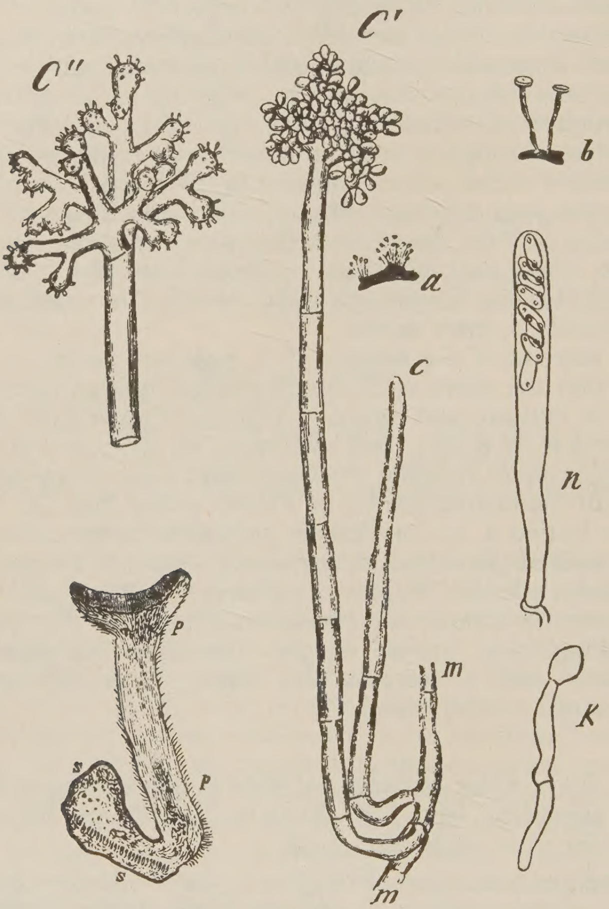
Elsewhere in this volume there are various references to *Peziza fuckeliana*, as well as two illustrations, none of which, however, can be considered as providing a recognizable description. Fig. 12 on p. 31 shows a thin section through a sclerotium magnified 390 times, and Fig. 17 on p. 39 shows a section through a sclerotium and young apothecium magnified 20 times, both of which are reproduced in the English translation (1887) of the enlarged later edition (1884) of this work. This first reference to *P. fuckeliana* by de Bary was cited as the original description of the species by Saccardo (1889) and by Rehm (in Rabenhorst 1896), but as de Bary merely stated that the fungus was to be described in detail elsewhere this entry is obviously a *nomen nudum.*

The first account of the biology of *P. fuckeliana* begins on p. 201. De Bary states that the sclerotia of this fungus are formed in tissues of dead vine leaves in autumn and winter. If they are placed on the surface of moist soil, either in a ripe fresh condition, or after several months' dry storage, they produce conidial fructifications in the course of twenty-four hours with all the characteristics of *Botrytis cinerea* Pers. If, however, the sclerotia are buried a few millimetres or a centimetre under the surface of the soil, stalked plate-like ascus-bearing cups are formed in summer instead of conidiophores. Numerous cultures from germinating ascospores gave only sclerotia without the formation of conidia. The question if the *Botrytis* conidiophores, known to grow out from sclerotial forms like *Sclerotium durum* and *S. bullatum* also belong to the *Formenkreis Peziza fuckeliana* was still to be decided.

1869. *A. de Bary. Ueber Schimmel und Hefe. in* Virchow und Holtzendorf's *Samml. gemeinverst. wiss. Vortr.* Serie IV, Heft 87 and 88, pp. 551–630. Berlin (Separate pagination 1–78).

This semi-popular account of fungi and yeasts with special reference to fermentation processes was made the occasion for fairly full descriptions of the biology of some typical moulds and yeasts: *Aspergillus glaucus*, *Botrytis cinerea*, *Mucor stolonifera*, *M. mucedo*, *Penicillium glaucum*, *Oidium lactis* and *Saccaromyces cerevisiae.* Nine pages are devoted by de Bary to *Botrytis cinerea* and *Peziza fuckeliana* and with his 'Fig. 2 *Botrytis cinerea*', reproduced here as Text-fig. 1, presumably forms the description of *Peziza fuckeliana* n.sp. to which a passing reference was made in 1866. The description is diffuse and its main points relevant to *Sclerotinia fuckeliana* are as follows.

Botrytis cinerea is widespread on many different substrata but de Bary definitely states that his description is confined to the one which is almost always to be found on dead, damp, fallen vine leaves. The development of conidiophores, their evanescent terminal bladders on which the conidia are formed and the formation of sclerotia in late autumn after the fall of the leaves are described. On the ground, if warm weather intervenes before the sclerotia are ripe, numerous clusters of *Botrytis* conidiophores may develop on the sclerotia (Text-fig. 1*a*) and the sclerotium subsequently shrivels. But if, after months of ripening, the sclerotia are placed on damp soil in summer or autumn a bundle of hyphae pushes through the rind of the sclerotium, elongates and spreads out at the free end as a flat plate-like disk on the free surface of which are produced paraphyses and asci (*n*) containing eight oval spores. When ripe the free end of the ascus bursts and the spores are scattered to a great distance. Small sclerotia produce only one ascophore, larger sclerotia two to four (*b*). The ascophore, when full



Figur 2. Botrytis cinerea.

a u. *b*. Natürliche Größe; Sclerotien, aus denen bei *a* Conidienträger, bei *b* Schlauchfrüchte hervorwachſen. *c*, *c′* Conidienträger (*c′* mit eben reifen Conidien) von dem Myceliumfaden *m* entſpringend (Vergr. etwa 200). *C″* Ende eines Conidienträgers mit dem erſten Beginn der Conidienabſchnürung auf den Zweigenden. *k* Keimende Conidie (Vergr. 300). — *d* (ſchwach vergr.) Durchſchnitt durch ein Sclerotium *s*, aus welchem ein ſehr kleiner Schlauchträger (*p*, *p*) hervorwächſt. *n* (Vergr. 390) Einzelner Sporenſchlauch mit 8 reifen Sporen.

Text-fig. 1. *Botrytis cinerea*.

grown, has a stalk one or more millimetres long and the diameter of the disk is one-third to three millimetres (seldom more). It is stated as a matter of special interest that in suitable media, such as on the damp injured surfaces of vine leaves, both conidia and ascospores put out germ tubes and grow directly into mycelial hyphae which can form conidiophores and finally sclerotia.

The mycelium and conidiophores on vine leaves are indistinguishable from the universally distributed mould on dead parts of plants of every kind. The sclerotia are also similar in form, but may be somewhat larger on other substrata, and produce masses of conidiophores like the form described on vine leaves. The stalked ascophores on the contrary have seldom been observed on other substrata, and when found are always very similar to those which originate in vine leaves, though differing from them in many details. 'It is possible that these various ascus fruit bodies belong to different but closely related species, although their sclerotial and conidial forms must all be recognized as *Botrytis cinerea*. This is why the description has been confined to the form on the vine whose development is well-known. Sclerotia are formed only on solid vegetable tissues such as leaves, pumpkins and thick stalks, while on delicate parts like flowers only mycelia and conidiophores are formed. The conidiophores, sclerotia and ascus-fruits were formerly regarded as distinct fungus species belonging to different genera, *Botrytis* (*B. cinerea*, *B. vulgaris*, etc.), *Sclerotium* (*S. durum*, *S. echinatum*). The former generic name is now applied to distinguish an organ or developmental stage which is present in many species; while the ascus-fruit belongs to the genus *Peziza*. It is the special form on vine leaves, which is named *Peziza Fuckeliana*.'

Thus, although not mentioned in the legend to 'Figur 2', the apothecium and ascus with ascospores are definitely stated in the text to be *P. fuckeliana* on vine leaves, the connexion being proved by observing the development of conidiophores on mycelia derived from ascospores.

Little more need be added to the description than to draw attention to the hairs on the stalk depicted below the apothecium and to give the measurements of the ascospores, which according to magnification indicated in the legend are about 12 × 5 μ while in the ascus. The single ascus is 130 μ long. The drawing is presumably made from the type specimen which is mounted in glycerine.

1869. *L. Fuckel. Symbolae Mycologicae. Beiträge zur Kenntnis der rheinischen Pilze. Wiesbaden. Jahrb. d. Nassauischen Ver. f. Naturk.* xxiii u. xxix, p. 330.

Fuckel erected the new genus *Sclerotinia*, the first-mentioned species of which (and generally taken as the type of the genus) is *S. Candolleana* (Lév.) Fuckel, and the second:

'*S. Fuckeliana* (de By.)—*Peziza Fuckeliana* de Bary. *Morph. u. Physiol.* p. 30. (Fung. integr.)—I. Mycelium quiescens, *Sclerotium echinatum* Fuckel, *E.F.N.* Nr. 215.—F. rh. 1478—An faulenden Blättern von Vitis vinifera, selten, im Frühling. Um Oesterreich. II. Fungus conidiophorus (?). *Botrytis cinerea* Pers. Syn. p. 690. Polyactis Autor. pl.—F. rh. 148.—Auf dem *Scle. ech.*, sehr häufig.

'Die Schlauchform sah ich noch nicht.'

On p. 458 Fuckel adds a note to p. 330: 'Nach de Bary's neuesten Untersuchungen ist die genetische Beziehung dieser hier zusammengestellten Entwicklungsstadien ausser allen Zweifel gestellt.'

1872. *A. de Bary.* On Mildew and Fermentation. *Quarterly German Magazine* (Berlin), II, 153–226 (separate pagination 1–76).

This is a literal English translation of de Bary's 'Ueber Schimmel und Hefe' 1869, and contains 'Figur 2. *Botrytis cinerea*', apparently reprinted from the original wood block. Many technical terms are incorrectly translated.

1875–6. *M. C. Cooke.* Carpology of Peziza. *Grevillea*, IV, 132.

The legend to Pl. LXV, fig. 281, reads: '*Peziza Fuckeliana.* D'By. fide Dr. Bary.'

The figure of a single ascus is apparently copied from de Bary's 1869 paper, but has in addition two ascospores lying outside the ascus. The ascus measures 140 μ, according to the scale given by Cooke at the start of this series of illustrations, while the spores are $13{\cdot}5 \times 5{\cdot}5\,\mu$.

1881. *R. Pirotta.* Sullo sviluppo della Peziza Fuckeliana de By. e della P. Sclerotiorum Lib. *Nuovo Giornale Botanico*, 13: 130. See *J. Microsc. Soc.* I, 235–40.

The author describes conditions under which the different stages are formed, but the paper contains no description of the fungus. It is stated incidentally that Brefeld, van Tieghem, Klein, Cornu, Tichomiroff and Schroeter have tried to confirm de Bary's assertion that *Botrytis cinerea* is the conidial stage of *Peziza fuckeliana.* Pirotta himself collected *Sclerotium echinatum* on fallen vine leaves and obtained *Botrytis cinerea*, and in one case six Peziza cups.

1884. *A. de Bary. Vergleichende Morphologie und Biologie der Pilze, Mycetozoa und Bakterien.* Leipzig.

This is usually referred to as the second edition of de Bary 1868, but actually is largely a new work. There are numerous references in it to a fungus referred to indiscriminately as *Peziza fuckeliana* and *Sclerotinia fuckeliana.* It is stated that conidiophores can arise directly on the mycelium growing from ascospores, and further that germinating conidia produce a mycelium with all the characters and products of the mycelium produced from ascospores, except that it inclines more to the production of conidiophores. Frequently 'abortive conidia' or 'spermatia' are formed, and there is a new illustration of the mode of formation of spermatia in *Peziza fuckeliana* (p. 264, fig. 116).

1886. *A. de Bary.* Ueber einige Sclerotien und Sclerotinien-Krankheiten. *Botan. Zeitg.* 22–27 (p. 377).

It is recorded that *Botrytis cinerea* is found only in association with *Sclerotinia fuckeliana*, and not with other species of *Sclerotinia.*

1887. *A. de Bary. Comparative morphology and biology of the fungi, mycetozoa and bacteria.* Oxford.

The English translation of the 1884 work; it apparently contains no further information.

1889. *P. A. Saccardo. Sylloge fungorum*, VIII, p. 196.

Sclerotinia fuckeliana. De Bary. *Morph. Pilz.* p. 30, *Fuck. Symb.* p. 330, Pirotta in *N.G.B.* l. 1881. p. 130—Stipitata, minuta, patellaris, ½-3 mm. lata, 5–10 mm. alta, flavo-brunnea ex Sclerotio echinato Fuck. nascens; ascis tereticlavatis, 130 = 12–13; sporidiis monostichis, ovoideis, hyalinis, 10 – 11 = 6 – 7.

Hab. in Sclerotio echinato ad folia putrida Vitis viniferae in Germania, Italia.

Status conidicus est *Botrytis cinerea* Pers.

This entry omits references to de Bary's paper of 1869, but the description given is apparently based on the description and figure in this work with the additional information on the colour of the apothecium, which does not seem to have been mentioned by de Bary.

1890. *W. Zopf. Die Pilze*, p. 472.

This work is remarkable for republishing 'Figur 2' from de Bary's 1869 paper and is also unique in citing this paper for the description of *Sclerotinia fuckeliana* instead of the book of 1866. The magnification of the ascus is stated to be '× 300' while in de Bary's paper it is given as '× 390', and this would make the ascus about 170 μ long, and the spores about 16 × 5·5 μ. From examination of the type specimen Zopf's magnification is evidently a misprint.

1896. *H. Rehm.* in Rabenhorst's *Kryptogamen Flora*, I, 3, p. 811.

The descriptions previously cited are apparently all based on de Bary's account and figure, but Rehm appears to have re-examined the fungus and to have based his description on new observations, and as a result his account differs in some respects from de Bary's. He says:

5408. *Scl. Fuckeliana* (De Bary).

Synon.: *Peziza Fuckeliana* De Bary (*Morph. Phys. der Pilze*, p. 30, fig. 12, p. 238).

Sclerotinia Fuckeliana Fuckel (*Symb. myc.* p. 330).

Exsicc.: Fuckel, *Fungi rhen.* 1478 (Sclerotium).

Apothecien meist einzeln, aus einem in dem Blattnerv gebildeten, später freien, länglichen oder runden, oft unregelmässigen, 2–4 Millim. langen, 1–2·5 Millim. breiten und dicken, aussen schwarzen, zuletzt glänzenden, feinwarzigen Sclerotium sich entwickelend, zuerst kuglig geschlossen, dann kelchförmig, rundlich sich öffnend und die zuletzt flach schüsselförmige, zart berandete Fruchtscheibe entblössend, mit einem cylindrischen, geraden oder gebogenen 2–10 Millim. langen, 0·1 Millim. breiten Stiel, aussen glatt, schwach bräunlich, trocken verbogen, 0·2–0·5 Millim. breit, wachsartig. Schläuche cylindrisch, oben abgerundet, 100–120 μ lang, 9–12 μ breit, 8 sporig. Sporen länglich-elliptisch, stumpf, einzellig mit je einem kleinen Oeltropfen in der Ecke, farblos, 9–11 μ lang, 5–6 μ breit, einreihig liegend. Paraphysen fädig, septiert, nach oben allmählich 5 μ breit, farblos. Gehäuse prosenchymatisch, schwach bräunlich. Jod bläut den Schlauchporus.

An faulenden Blättern des Weinstockes im Rheingau, bei Halle (Winter), Grünberg in Schlesien (Schröter).

In the course of a long discussion of *Sclerotinia fuckeliana* Rehm adds: 'Die Beschreibung geschah nach den in meinem Besitz befindlichen, aus dem *Sclerotium echinatum*, wie es Fuckel l.c. nannte, durch Cultur erzogenen Examplaren Winter's.'

1902. *Annie Lorrain Smith and Carleton Rea.* Fungi New to Britain. *Trans. Brit. mycol. Soc.* XXXV.

Recording a specimen grown from sclerotia of a fungus causing a disease of gooseberry bushes in Hereford, April 1902, the authors translate and slightly simplify Rehm's description quoted above. Their specimen is in Herb. Mus. Brit.

1903. *Annie Lorrain Smith.* A disease of the gooseberry. *J. Bot.* XLI, 19–23.

This gives a further account of the same outbreak of *Botrytis* on a plantation of seven-year-old gooseberries in the Hereford district. In addition to conidia 8–11 × 4–6 μ, 'a *Peziza* also grew from one of the sclerotia in the damp chamber'. This specimen was said to resemble *Sclerotinia fuckeliana*; ascospores remaining in the ascus were oval and measured 10–12 × 6 μ.

1905. *Gy. de Istvanffi.* Études microbiologiques et mycologiques sur le rot gris de la vigne (*Botrytis cinerea-Sclerotinia Fuckeliana*). *Ann. de l'Inst. Cent. Ampélologique Roy. Hongrois*, III, 184–360, figs. 1–211.

Istvanffi made a thorough re-examination of sclerotial, conidial and apothecial stages, and described the development of all stages in detail. The asci are stated to be only 60 × 6 μ, containing from 5–8 spores measuring 9 × 4 μ, apparently described from microtome sections in paraffin wax stained with Heidenhain's haematoxylin. Apparently apothecia were not obtained from sclerotia grown in pure culture.

1911. *P.A. Saccardo. Sylloge fungorum*, XX. Index Iconium Fungorum, p. 764.

Under '(*Sclerotinia*) *Fuckeliana* de Bary', Saccardo lists twenty-two sources of illustrations, but the figure from de Bary's papers of 1869 and 1872, copied by Zopf 1890 and reproduced here, is omitted.

1927. *Said Kharbush.* Évolution nucléaire du *Sclerotinia Fuckeliana* de Bary. *Bull. Soc. Botan. de France*, LXXIV, 257–67.

Kharbush states that in artificial culture some sclerotia give sterile apothecia of *Sclerotinia*, others tufts of *Botrytis cinerea* conidiophores. A cytological study was made of fertile apothecia and ascospores were found to be oval or oblong-elliptical, 9 × 4 μ, and on germination they gave a mycelium producing *Botrytis* conidiophores and sclerotia.

1945. *H. H. Whetzel.* A synopsis of the genera and species of the Sclerotiniaceae, a family of stromatic inoperculate Discomycetes. *Mycologia*, XXXVII, 648–714.

In this posthumous work the editor, Dr H. M. Fitzpatrick, records that in 1930 Whetzel collected a single apothecium growing from a sclerotium attached to a grape cane, and that his studies indicated that it was *Sclerotinia fuckeliana*. Ascospores from it gave a culture of the *Botrytis cinerea* type. It is unfortunate that de Bary's specimens were not available to Whetzel for use in his studies, because, as Fitzpatrick points out, Whetzel expected to designate *Sclerotinia fuckeliana* as the type species of his new genus *Botryotinia*. The species chosen as the type of the genus was *B. convoluta* Drayton, and the new combinations *B. fuckeliana*, *B. porri* and *B. ricini* were established.

de Bary's specimens of Peziza Fuckeliana

The specimens of *P. fuckeliana* in de Bary's collection of slides of cryptogams in the British Museum (Natural History) consist of sections mounted in glycerine and labelled in de Bary's handwriting.

(1) '*Peziza Fuckeliana* 22/10/64.' This slide contains three vertical sections through an apothecium and stalk. The most complete section is illustrated in Pl. I, fig. 1, and shows a disk 1·8 mm. diam., with a hymenial layer approximately 140 μ thick. The stem is cut obliquely and is about 0·6 mm. wide at the top. The fine hairs formed of single hyphae, which clothe the stem, are visible in some places. These hairs are shown at higher magnification in Pl. III, fig. 1.

Pl. II, fig. 1 shows the right-hand margin of the apothecium at a higher magnification. The bi-guttulate ascospores, approximately $8{\cdot}5\text{–}10 \times 3{\cdot}5\text{–}4\,\mu$ are clearly shown.

Pl. II, fig. 2 illustrates a portion of one of the other sections on this slide where the layer of asci has been squashed and shows paraphyses.

(2) '*Sclerotium peziza fuckeliana* (Vitis) Fbg [= Freiburg?] Febr. 62. Peziza, Sept. 62.' This slide contains three transverse sections through sclerotia. One of these is illustrated in Pl. III, fig. 3, and traces of xylem of the host plant can be seen embedded in the sclerotium.

In addition to these earlier sections of sclerotia, there are six other slides of sclerotia labelled: '*Peziza fuckeliana* 1/8/82.'

(3) '*Peziza fuckeliana.* Spermatium? cult .9/82.' A typical portion of this slide is illustrated in Pl. III, fig. 2, and shows hyphae, spermodochia and spermatia. Probably, this slide was used in making the drawing for fig. 116 of de Bary's book of 1884.

These studies were begun at Seale-Hayne Agricultural College, Newton Abbot, and completed at Rothamsted Experimental Station, Harpenden. Help in tracing literature and slides given by Dr G. R. Bisby, Dr J. Ramsbottom, and Dr R. Eric Taylor is gratefully acknowledged. The writer also wishes to thank the Trustees of the British Museum (Natural History) for permission to publish the photographs, Pls. I–III.

SUMMARY

Interest in *Sclerotinia fuckeliana*, described by de Bary as the perfect stage of *Botrytis cinerea* on vine, is revived by the production of apothecia in pure cultures by Drayton and Groves. The identification of these apothecia has been hindered by lack of information on de Bary's fungus, the reference usually cited as a description being a *nomen nudum*.

The various descriptions of this fungus are cited, including a detailed account and figure by de Bary, which appear hitherto to have been overlooked. Photographs are also given of de Bary's slide of *Sclerotinia fuckeliana* from the British Museum (Natural History), which evidently constitutes the type specimen. The information now available should help to determine the position of apothecia produced in pure culture.

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EXPLANATION OF PLATES

Label on Slide. '*Peziza fuckeliana* 22/10/64.' (Three vertical sections through apothecium, mounted in glycerine.)

PLATE I

Fig. 1. General structure of apothecium. × 83.

PLATE II

Fig. 1. Margin of same apothecium showing asci with 8 biguttulate ascospores. × 380.
Fig. 2. Portion of another section which has been squashed, showing paraphyses. × 380.

PLATE III

Fig. 1. Hairs on stalk of apothecium (just visible on right-hand side of Pl. I, fig. 1). × 380.

Label on Slide. '*Peziza fuckeliana*. Spermatium? cult. 9/82.'

Fig. 2. Groups of microconidial (spermatial) sporodochia. × 380.

Label on Slide. '*Sclerotium pezizae fuckeliana* (Vitis). Fbg. 62. Peziza, Sept. 62.'

Fig. 3. Transverse section of sclerotium with parts of host plant tissue, and traces of xylem embedded in sclerotium. × 195.

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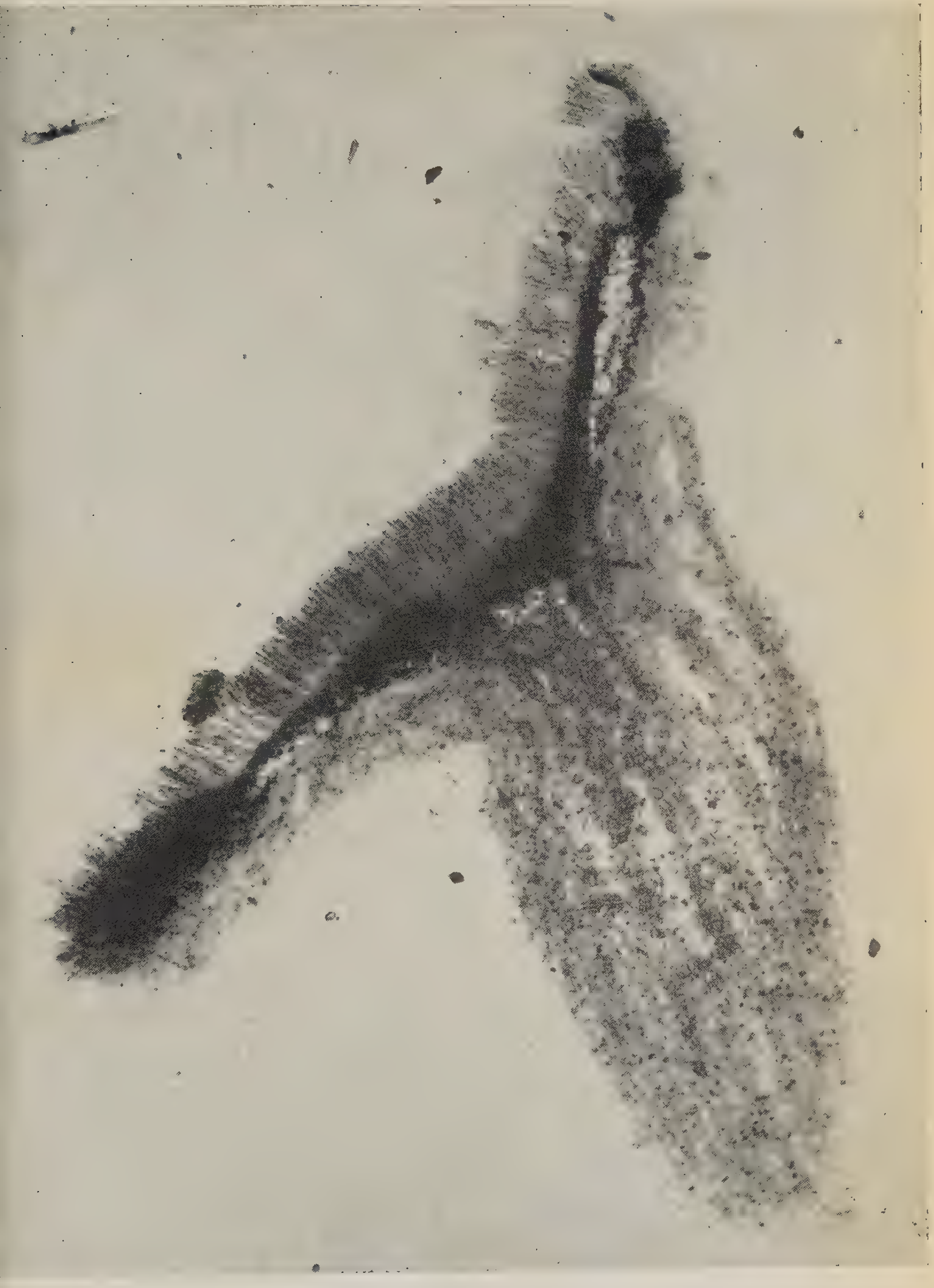
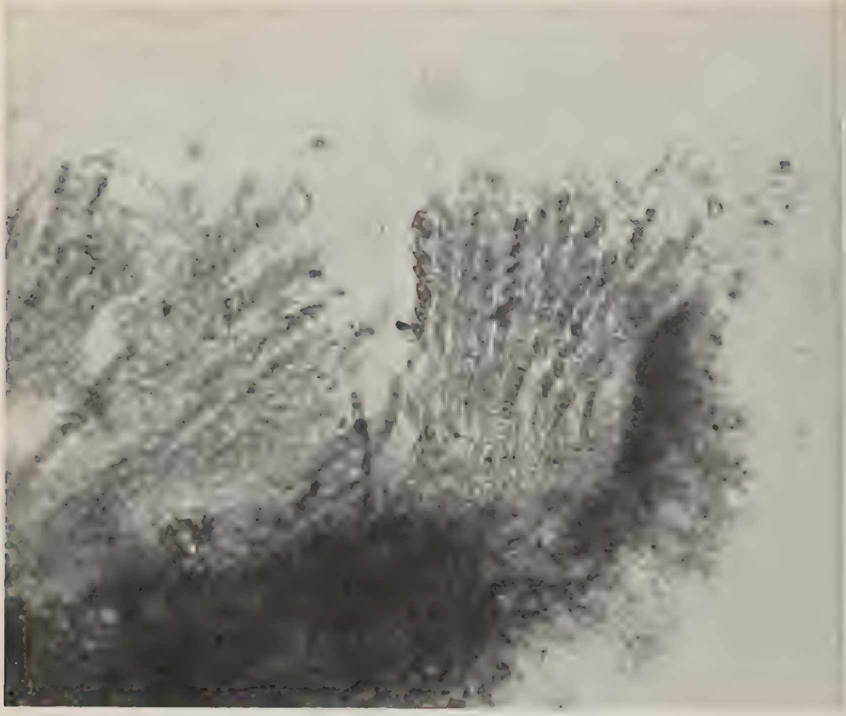


Fig. 1



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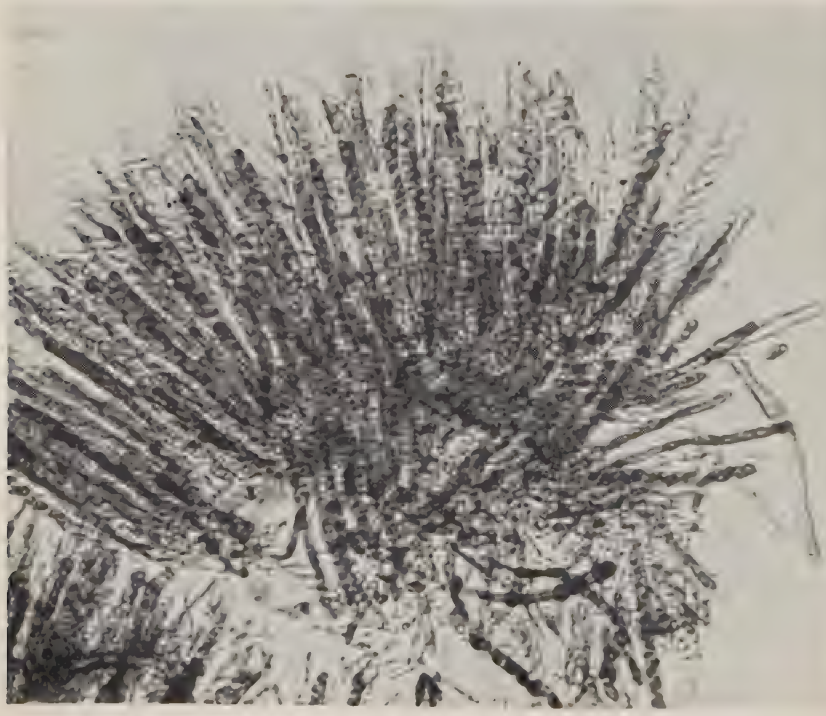

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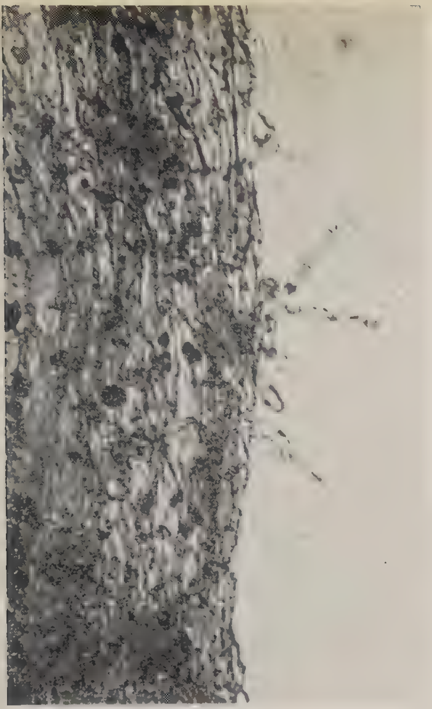

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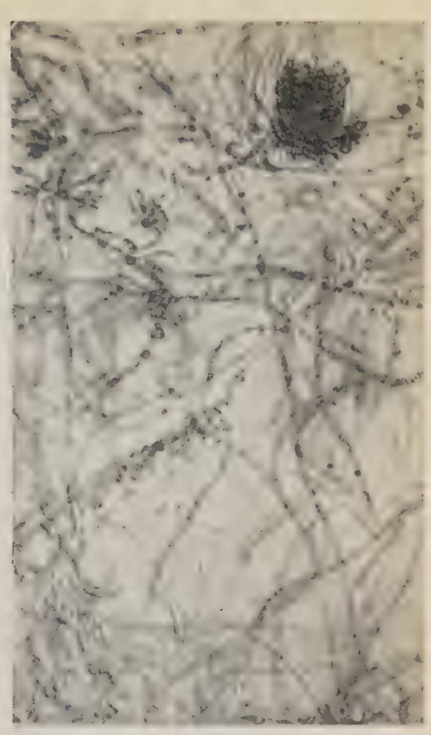

Fig. 2

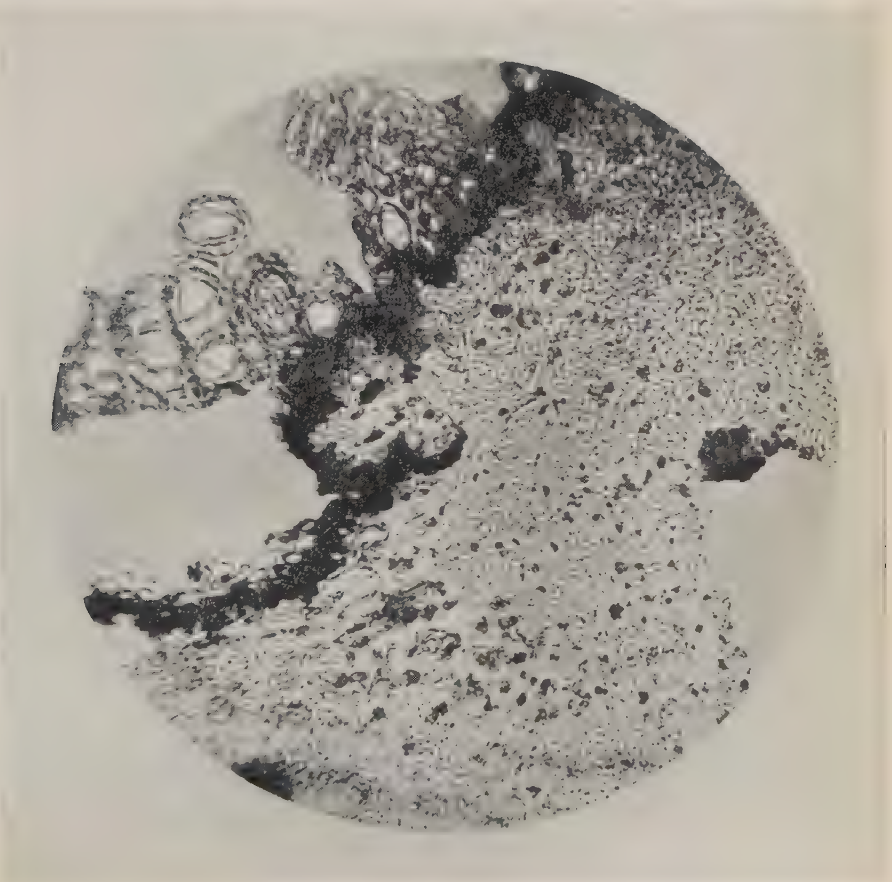

Fig. 3

THE OPERATION OF THE PUFF-BALL MECHANISM OF *LYCOPERDON PERLATUM* BY RAINDROPS SHOWN BY ULTRA-HIGH-SPEED SCHLIEREN CINEMATOGRAPHY

By P. H. GREGORY
Agricultural Research Council Research Officer at Rothamsted Experimental Station, Harpenden, Herts

(With Plate IV and 1 Text-figure)

On blowing across the top of a fruit body of *Lycoperdon perlatum* it is easy to evoke a cloud of spores, but the velocity of the air stream under these conditions is high. Preliminary tests in a small wind tunnel indicated that wind of ordinary speeds is not very effective in removing spores through the apical mouth. The puff-ball mechanism can, of course, be operated mechanically by animals, but emptied peridia are found in places where the number of blows received from animals is unlikely to be sufficient to account for discharge. Other natural agencies were therefore sought, which might operate the puff-ball mechanism in those species such as *L. perlatum*, *L. pyriforme* and *L. echinatum* which dehisce by a central apical pore*. The studies by Buller (1942) on the splash-cup mechanism of the Nidulariaceae (apparently first discovered by Martin, 1927) suggested that raindrops might also be capable of operating the puff-ball mechanism.

Drops of water were released from a pipette placed over a mature fruit body of *L. perlatum* in the laboratory. Impact of the drop with the papery, flattened top of the peridium, to the side of the ostiole, was instantly followed by the ejection of a small puff of spores to a height of two or three centimetres. Under laboratory conditions vortex rings were sometimes formed. Observations out of doors during rain showed that the mechanism was also operated by raindrops under natural conditions. Fine misty rain did not produce a puff visible in dull diffuse daylight, but thunder rain was excellent. Rain-drip from leaves of trees during fine rain consists of relatively large drops which made the puff-ball 'smoke' as though on fire. *L. perlatum* var. *lacunosum*, *L. pyriforme* and *Geaster umbilicatus*, were also easily operated by raindrops.

The mode of action of the drop was analysed by ultra-high-speed Schlieren cinematography, using a large mature fruit body of *L. perlatum* collected on Harpenden Common in November 1944. The camera used was the Kodak Type III high-speed cine camera described by Jones (1944), fitted with a tuning fork controlled time base which photographs time marks on the edge of the film at 0·001 sec. intervals (Eyles, 1943). The optical system is illustrated in Text-fig. 1, and is similar to that described originally by Taylor and Waldram (1933).

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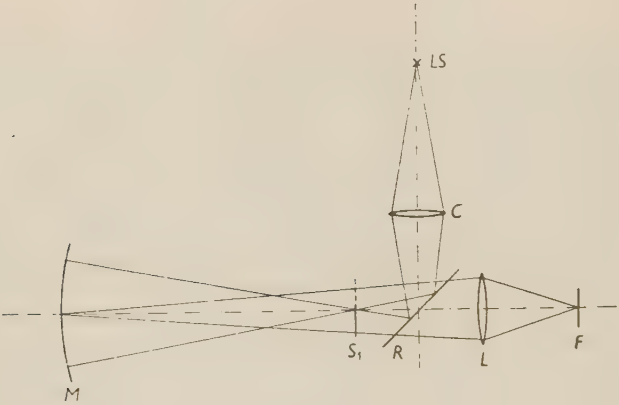
* In such forms as *L. saccatum* and *L. giganteum* the endoperidium peels away at maturity exposing the gleba directly to the action of wind and rain. Spore dispersal in this type has been well described by Falck (1909) in a species referred to as '*L. pyriforme*' but probably identical with our *L. saccatum*.

The concave spherical mirror condenser *M* is 12 in. in diameter and its surface is optically worked to a high degree of accuracy and sputtered with aluminium to give it high reflectance. An image of the 500 W. compact source high-pressure mercury vapour lamp LS is thrown by the condense *C*, corrected for spherical aberration, from the surface of the plane mirro *R* to the centre of curvature of the mirror *M*. The mirror *R* is rhodium-plated to reflect about 50 % of the incident light. From the centre o curvature of the concave mirror *M*, the light proceeds to its surface and i reflected back along its own path through the plane mirror *R* into the



Text-fig. 1.

camera·lens *L*, forming an image of the concave mirror *M* at its foca plane *F*, which is the plane of the film in the camera. In this way, a extended field of uniform illumination of high brightness is produced. Th brightness is, in fact, equal to that of the surface of the light source itself reduced only by the reflexion losses at the surfaces of the optical system The photographs taken under these conditions are therefore shadow photographs of the subject placed close to and in front of the concave mirror *M*

The sensitivity of the optical system described above can be increase by reducing the effective size of the light source LS. This is convenientl carried out by introducing a knife-edged diaphragm S_1 at the centre o curvature of the concave mirror *M*. An image of this diaphragm is thu formed at the same point by reflexion from the concave mirror *M*. If th position of the knife edge S_1 is adjusted properly, it can be made to for a fine parallel slit with its own image, reducing effectively the size of th light source LS in a horizontal plane and thereby increasing the resolvin power of the system. This so-called 'Schlieren' system of illumination ca be made so sensitive that small variations in the refractive index of the ai or other transparent media in front of the concave mirror *M* will be made visible as shadows in the focal plane of the camera lens *L*. In the case of the photography of spore clouds with which we are dealing, it is doubtful whether the system was adjusted to the high sensitivity required to show up eddies in the air disturbed by ejection of the spores. The spore clouds were, however, effectively larger on the record when photographed with the 'Schlieren' or knife-edged diaphragm in place, because of the increased resolving power of the optical system under these conditions which would make it possible to record parts of the cloud containing relatively few spores.

For calibration a series of photographs was first obtained with a graticule with divisions 1 cm. apart, made on a photographic plate, superimposed in front of the concave mirror. Later series were made without the graticule and with the knife-edge diaphragm in position. Examples of these are shown in Pl. IV, figs. 1–9.

Analysis of a number of runs, made at from 600 to 1770 pictures/sec., shows that the water drops. of 5·0–5·2 mm. diameter, had a velocity immediately before impact of 440–470 cm./sec. For approximately 0·002 sec. after impact the only visible change (not shown in Pl. IV, fig. 3 owing to high contrast) was the development of the splash in the form of the usual corona with radial arms. At 0·003 sec. a puff of spores began to emerge from the ostiole, reaching a height of 0·5 cm. in from 0·004 to 0·008 sec., and 1 cm. in from 0·01 to 0·015 sec. The initial velocity of the puff was from 50 to 400 cm./sec. The cloud slowed down on reaching a height of about 1·5–2·0 cm. at about 0·02–0·04 sec. after impact. When the maximum height was reached the cloud was diffused and carried away by air currents.

No observable deformation of the peridium occurred during or after impact. Presumably, however, the thin surface of the peridium is momentarily invaginated against the supporting layer of the capillitium, leading to the ejection of air and spores through the ostiole. The original shape of the peridium is apparently restored by pressure of the capillitium.

Natural raindrops vary from about 0·2 to 5·0 mm. diameter (Simpson, 1941), and drops of different size have different terminal velocities and therefore strike the peridium with different kinetic energies. The minimum energy required to operate the mechanism was tested in the laboratory with a beam of light directed horizontally across the top of the peridium to show the puff of spores clearly. It was not convenient to produce drops of less than 2·5 mm. diameter, and instead of using various sized drops at their terminal velocity, a standard sized drop was released from different heights. By finding the minimum height required by the drop to gain sufficient velocity to operate the puff-ball, the minimum kinetic energy required can be calculated. An estimate can then be made of the size of the minimum natural raindrop, falling at terminal velocity, required to operate the puff-ball. The kinetic energy of the drop is given by $\frac{1}{2}mv^2$ (where m = mass in g., v = velocity in cm.).

It was found that drops 2·5 mm. diameter would operate the fruit body of *L. perlatum* when falling from a height of 5–7 cm. or more, corresponding to a kinetic energy of about 40–60 ergs. Observed terminal velocities of raindrops of various sizes are given by Lenard (1904), and somewhat higher velocities were reported by Ellison (1944). Calculations from Lenard's data show that a drop of 1 mm. diameter would have a kinetic energy of about 48 ergs and so would be about the minimum size required to operate the mechanism. Lenard found that in ordinary general rain in Switzerland, about 25 % of the drops were 1 mm. or more in diameter, and 42 % raindrops of all types examined were in this category. Laws and Parsons (1944) give somewhat similar data from the United States, while Kelkar (1945) from Poona reported the following proportions of raindrops of 1 mm. diameter and over: drizzle, 0·4; light shower, 18·8; moderate shower, 69·2; heavy shower, 90·6; very heavy shower, 35·1 %. From Lenard's data the number of drops striking 1 sq.cm. of horizontal surface during the fall of 1 cm. of 'ordinary rain' would total about 9000, one-quarter of which would be capable of operating the mechanism. A large fruit body of *L. perlatum* with a horizontal surface of 10 sq.cm. might be operated over a quarter of a million times during a year's exposure in a locality with a rainfall of 100 cm. (39 in.).

The number of spores emitted will obviously depend on a number of factors, including: kinetic energy of the drop, position of hit and deformation of the peridium, size of the puff-ball and number of spores remaining. In order to get some indication of the number of spores ejected as a result of raindrop impact a typical fruit body of *L. perlatum* of approximately 2 cm. diameter, collected near Harpenden, in November 1944, was chosen for test. The puff-ball was anchored at the bottom of a deep beaker whose mouth was covered by a card with a central hole through which drops of water of approximately 4·2 mm. diameter (0·038 g.) were allowed to fall on the top of the moistened peridium. In each test ten drops were allowed to strike the peridium, and after allowing the spores to settle, the floor and sides of the beaker were washed out with water containing a wetting agent. The number of spores liberated was then estimated from samples in a haemocytometer slide.

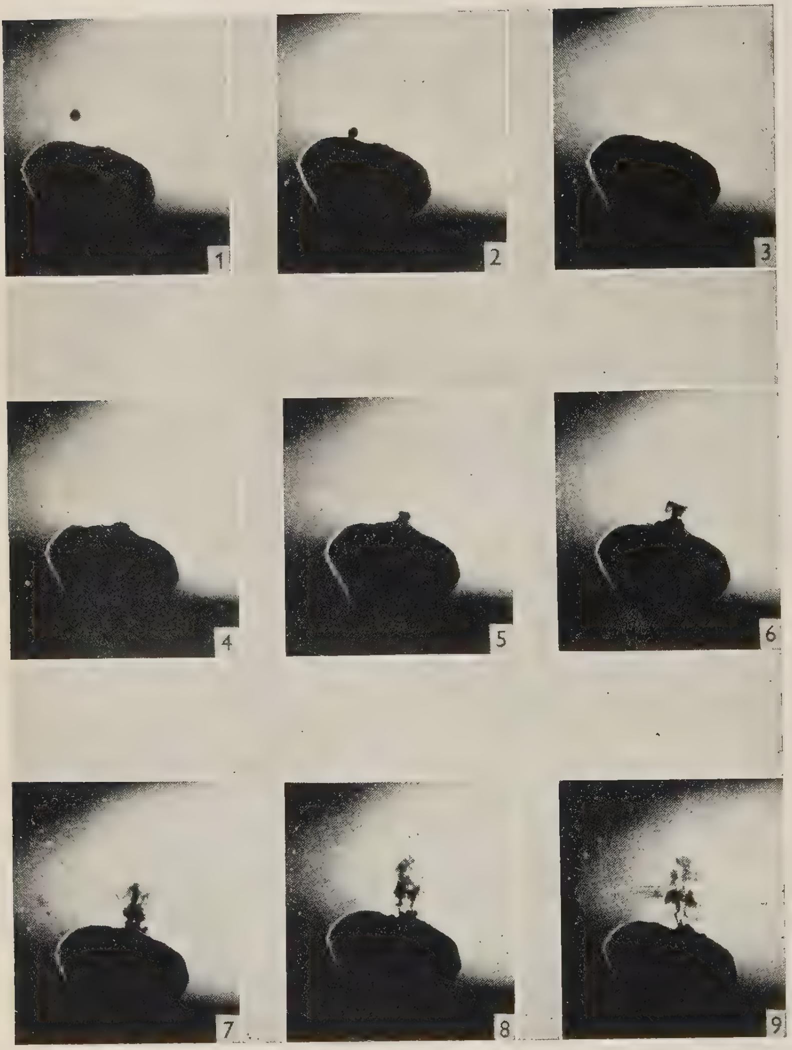
Two series of tests were carried out with drops falling from each of two heights. The mean number ejected with a drop falling 43 cm. was $0·99 \times 10^6$ spores, and with a drop falling 130 cm., $15·6 \times 10^6$ spores. The total number of spores remaining in the puff-ball was estimated as 4460×10^6.

The outside of the mature peridium is easily wetted by rain. When wet it darkens, swells and becomes more pliable. Wetting of the endoperidium does not hinder puffing, as the wall is waterproof and the spores remain dry. In one test a fruit body of *L. perlatum* was immersed in water to just below the ostiole and would still puff freely when removed and tested after three days.

Raindrop impact must therefore be one of the most efficient agents in liberating spores from the *L. perlatum* type of puff-ball. The relative importance of wind and raindrop impact awaits investigation.

SUMMARY

Spore discharge from puff-balls of the *Lycoperdon perlatum* type can be brought about by impact of water drops with the flattened papery top of the endoperidium. Raindrops of 1 mm. diameter or over, and rain-drip



Figs. 1–9

from trees are adequate to operate the mechanism. Analysis of the operation by ultra-high-speed photographs shows that the puff reaches a height of a centimetre in approximately one-hundredth of a second after impact. The velocity of the puff on emerging from the ostiole is of the order of 100 cm./sec.

The endoperidium of *L. perlatum* is normally water-proof and ejection continues under humid conditions. Estimates, made from meteorological data, show that a fruit body must be operated many thousands of times in a season. It is concluded that raindrop impact is efficient in liberating spores from *L. perlatum*.

These observations were incidental to studies on the dispersal of plant pathogens. The photographs were possible only because of the resources made available by the Kodak Research Laboratories, Harrow. In particular the writer is indebted for the collaboration of Mr E. D. Eyles who suggested and successfully carried out the analysis of the spore discharge mechanism by ultra-high-speed Schlieren cinematography.

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EXPLANATION OF PLATE IV

Stills from ultra-high-speed film showing impact of water drop with fruit body of *Lycoperdon perlatum* (¼ of natural size).

Fig. 1. Drop 5·0 mm. diameter falling with velocity of 440 cm./sec. Time: −0·00167 sec.
Fig. 2. Impact of drop on peridium to left of ostiole. Time: zero.
Fig. 3. After impact but before emergence of puff (splash corona not shown in print). Time: +0·00167 sec.
Fig. 4. Puff of spores beginning to emerge through ostiole with velocity of 140 cm./sec. Time: +0·00334 sec.
Fig. 5. Stages in diffusion of puff. Time: +0·0050 sec.
Fig. 6. Time: +0·0067 sec.
Fig. 7. Time: +0·0020 sec.
Fig. 8. Time: +0·032 sec.
Fig. 9. Time: +0·046 sec.

(*Accepted for publication 2 June* 1947)

STUDIES ON BRITISH CHYTRIDS

IV. *CHYTRIOMYCES TABELLARIAE* (SCHRÖTER) N.COMB. PARASITIZED BY *SEPTOSPERMA ANOMALUM* (COUCH) WHIFFEN

By HILDA M. CANTER, Ph.D.
The Freshwater Biological Association, Wray Castle, Ambleside, and Department of Botany, Birkbeck College, University of London

(With 3 Text-figures)

A fungus believed to be *Phlyctidium tabellariae* Schröter (1897) was found parasitizing *Tabellaria flocculosa* (Roth) Kütz in a temporary mud pool bordering the northern edge of Blelham Tarn bog near Wray Castle, during January 1947 and three months later on *T. fenestrata* (Lyngbye) Kützing in the plankton of Crummock Water, Buttermere and Bassenthwaite in the English Lake District. Specimens on *T. fenestrata* were scarce and the following description of the chytrid is based entirely on material collected from Blelham bog. After the latter material had been kept in the laboratory for a week, *Chytriomyces tabellariae* itself was attacked by the hyperparasite *Septosperma anomalum* (Couch) Whiffen.

I. **Chytriomyces tabellariae** (Schröter) n.comb.

The spherical, uniguttulate zoospore settles on a host cell, encysts, and by the development of an extramatrical germ tube of varying length (up to 13 μ long) is carried above the surface of the diatom (Fig. 1*c–e*, *g*, *j*). In some specimens this extramatrical portion is difficult to observe, and in a few it appears to be absent. The germ tube having entered the diatom cell forms a little branched rhizoidal system of limited extent, which does not taper (Fig. 2*a–c*), and is usually only visible after staining. It is almost certain that *C. tabellariae* shows a similar type of development to *Chytridium schenkii* (Schenk) Scherffel, *C. appressum* Sparrow, and others, in which a part only of the zoospore wall enlarges to form the sporangium, the remainder persisting as an appendage. In *Chytriomyces tabellariae* a part of the zoospore grows out to form a more or less oval sporangium, while the unexpanded portion persists as a swelling to which the extramatrical germ tube is attached. This swelling does not thicken as in *Chytridium schenkii* and may possibly represent a part of the already expanded zoospore which failed to develop further when the unilateral expansion began to form. When the sporangium is viewed from above this spherical portion cannot be seen (Fig. 1*i–k*). The changes in the protoplasm during development are similar to those described for the majority of chytrids. Before the sporangium is mature the portion of the wall forming the operculum is well marked (more especially in the larger specimens, Fig. 1*i*). It occupies a position

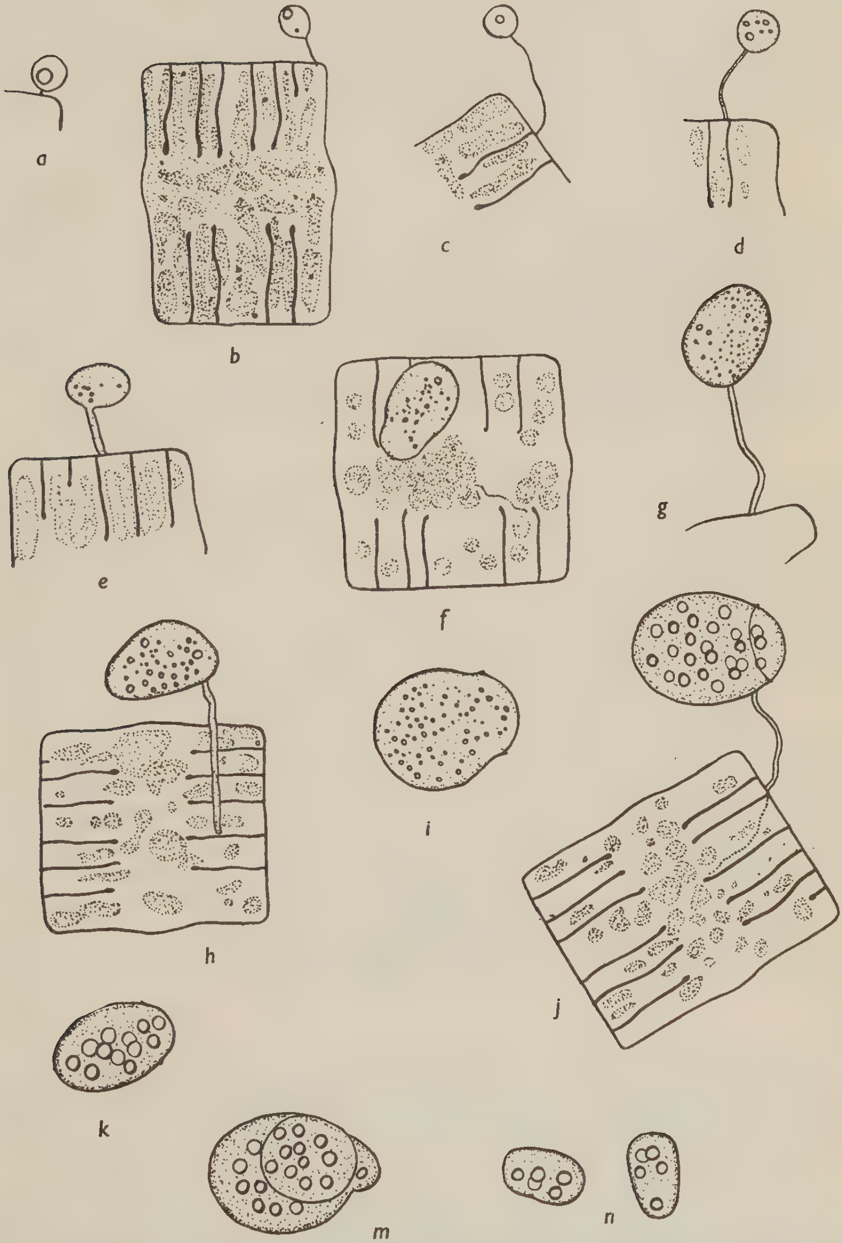

Fig. 1. *Chytriomyces tabellariae*. *a–h*, stages in early development of thallus. *i*, immature sporangium, operculum clearly visible. *j–m*, mature sporangia. All × 1400.

immediately above the basal spherical portion. The mature sporangia vary in size from 4·3–9 μ high × 7·6–15 μ broad and contain from 5 to 30 zoospores according to their size (Fig. 1 *n*, *m*). The actual moment of dehiscence was never observed, and thus it is not known whether the zoospores emerge singly or in a mass. The zoospores are spherical, 3 μ in diameter, with a conspicuous oil globule and single posterior flagellum.

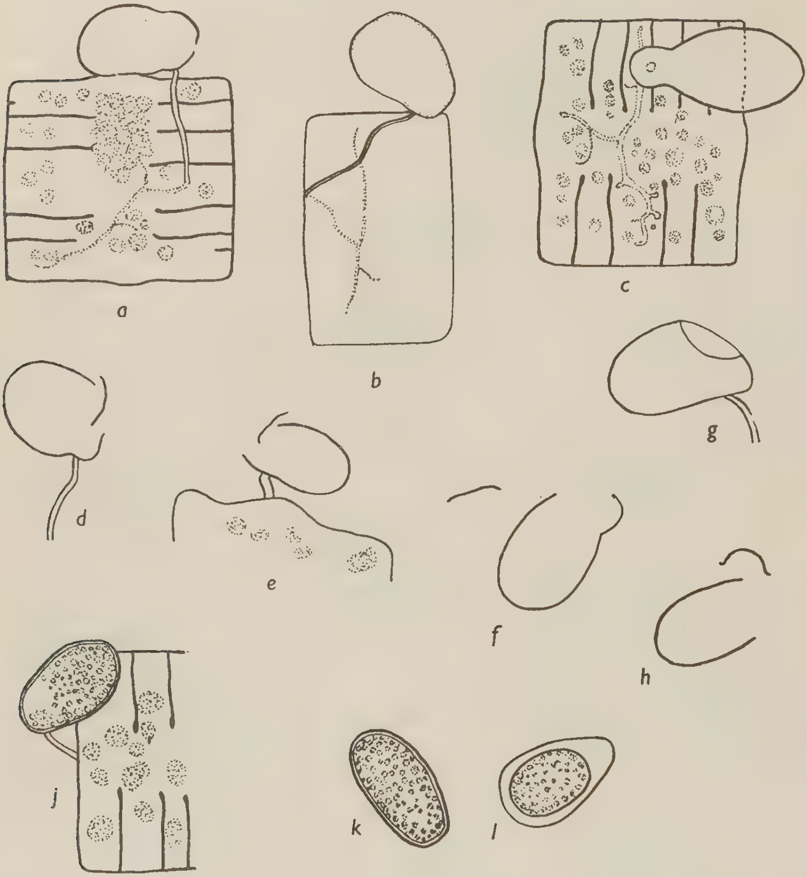

Fig. 2. *Chytriomyces tabellariae*. *a–c*, stained specimens showing full extent of rhizoidal system. *d–h*, empty sporangia. *j*, *k*, mature resting spores. *l*, resting spore formed by rounding off of protoplasm of the sporangium. All × 1400.

The convex operculum, 4·3–8 μ in diameter, often remains adherent to the empty sporangium or in its vicinity. Owing to the spherical unexpanded portion of the sporangium, the side of the wall from which the germ tube arises is longer than the opposite wall (Fig. 2 *d–f*), and it is this longer side which is measured in determining the breadth of the sporangium, while the height is taken in the mid-region.

A few resting spores were found as the material became moribund. They are borne as the sporangia and appear to be asexually formed. The mature resting spore (Fig. 2*j*, *k*) is oval, 12–9·9 μ broad × 6–5·7 μ high, with a thick, smooth wall and it contains numerous small globules. The rhizoidal system is similar to that formed by the sporangium, but the unexpanded portion of the latter was not seen. In some specimens (Fig. 2*l*) the resting spore seemed to be formed by the rounding off of the protoplasm of the sporangium and the subsequent secretion of a thick wall. Its method of germination is unknown.

Phlyctidium tabellariae has not been found, since its original description in 1897 by Schröter, on *Tabellaria fenestrata* (Lyngbye) Kützing var. *asterionelloides* Grunow. Schröter's description of this chytrid is incomplete and his figures are very small. Nevertheless, the British material agrees in the shape and thick-walled nature of the sporangium, in its basal area of dehiscence, point of origin of the extramatrical stalk, as well as in the nature of its host. It is thus suggested that these two organisms are synonymous, but in view of its operculate nature it is necessary to transfer this species to an operculate genus.

At present the operculate series of monocentric chytrids contains one genus, *Chytriomyces*, in which both the zoosporangia and asexually-formed resting spores are extramatrical and formed by enlargement of the zoospore body. There are four species; *C. aureus* and *C. hyalinus* Karling (1945), *C. nodulatus* Haskins (1946) are saprophytes on insect exuvieae, grass leaves, etc., while *C. spinosus* Fay (1947) is not markedly chitinophilic and grows more readily on carbohydrate substrata. In general, the sporangia are spherical, with an extensive, much branched rhizoidal system and the subsporangial swelling is not a constant feature in any one species.

It is clear that the fungus on *Tabellaria* differs from members of *Chytriomyces* in the method of development of the sporangium and in the nature of its rhizoidal system. If the operculate nature of *Rhizophydium echinatum* (Dang) Minden & Fischer, a parasite on *Glenodinium cinctum* is confirmed, then this fungus with its epibiotic sporangia and asexually-formed resting spores, both developed from enlargement of the zoospore, would show affinities with *Chytriomyces tabellariae*. It is proposed to include the fungus here described, which is identified with *Phlyctidium tabellariae*, in the genus *Chytriomyces* and the name becomes, therefore, *C. tabellariae* (Schröter) n.comb.

II. *SEPTOSPERMA ANOMALUM* (COUCH) WHIFFEN

Septosperma anomalum (*Phlyctidium anomalum*) was originally described by Couch (1932) parasitizing *Phlyctidium bumilleriae* Couch. Later Whiffen (1942) discovered a similar form growing on *Rhizophydium macrosporum* Karling and in view of the septate nature of the resting spore erected a new genus, *Septosperma*, to include these species, which have only been recorded from America. The British material (Fig. 3*a*–*q*) agrees well with *S. anomalum*. The sporangia are sessile, ovoid or ellipsoid, 6·6–12 μ high × 3·8–6·1 μ broad, and possess a small endobiotic, bulbous disk. Small sporangia produce about eight zoospores while up to thirty are formed in large ones. The zoospores are spherical, 2·3 μ in diameter, with a small oil globule and posterior flagellum. The resting spores, 6·6–12·4 μ high × 3·3–4·3 μ broad, agree with those formerly described. It is of interest that the hyperparasite attacks both young (Fig. 3*c*) and mature sporangia, in which the oil globules of the zoospores are delimited (Fig. 3*b*), but never mature resting spores. Infected specimens never reached maturity or as far as is known, liberated zoospores.

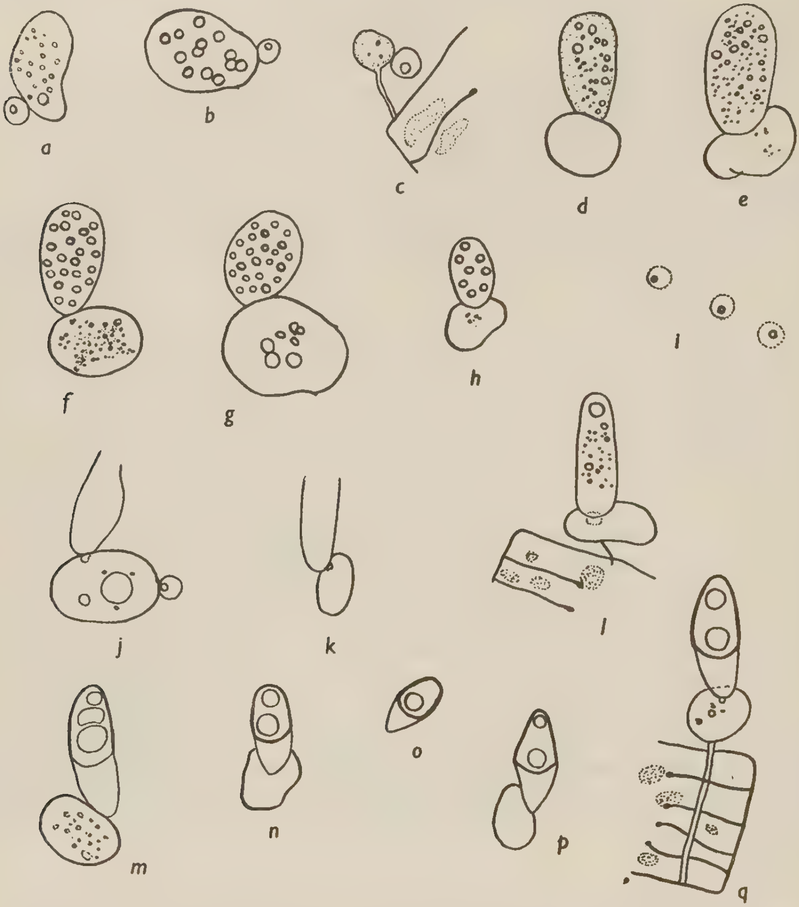

Fig. 3. *Septosperma anomalum.* *a–c*, zoospores attacking sporangia of *Chytriomyces tabellariae.* *d*, *e*, immature sporangia. *f–h*, mature sporangia. *i*, zoospores. *j*, *k*, empty sporangia, rhizoidal disk visible inside host sporangia. *l*, young resting spore. *m–q*, mature resting spores. All × 1400.

Summary

Two parasitic chytrids, both new records for Great Britain, are described. *Phlyctidium tabellariae* Schröter is found to be operculate and is transferred to the genus *Chytriomyces* Karling; the name becomes *C. tabellariae* Schröter n.comb. *Septosperma anomalum* (Couch) Whiffen was found parasitizing *C. tabellariae.*

My thanks are due to the Director of the Freshwater Biological Association for the use of a laboratory in which this work was done and to Prof. C. T. Ingold for reading the manuscript.

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STUDIES ON BRITISH CHYTRIDS

V. ON *OLPIDIUM HYALOTHECAE* SCHERFFEL AND *OLPIDIUM UTRICULIFORME* SCHERFFEL

By HILDA M. CANTER
The Freshwater Biological Laboratory, Wray Castle, Ambleside
and Department of Botany, Birkbeck College, University of London

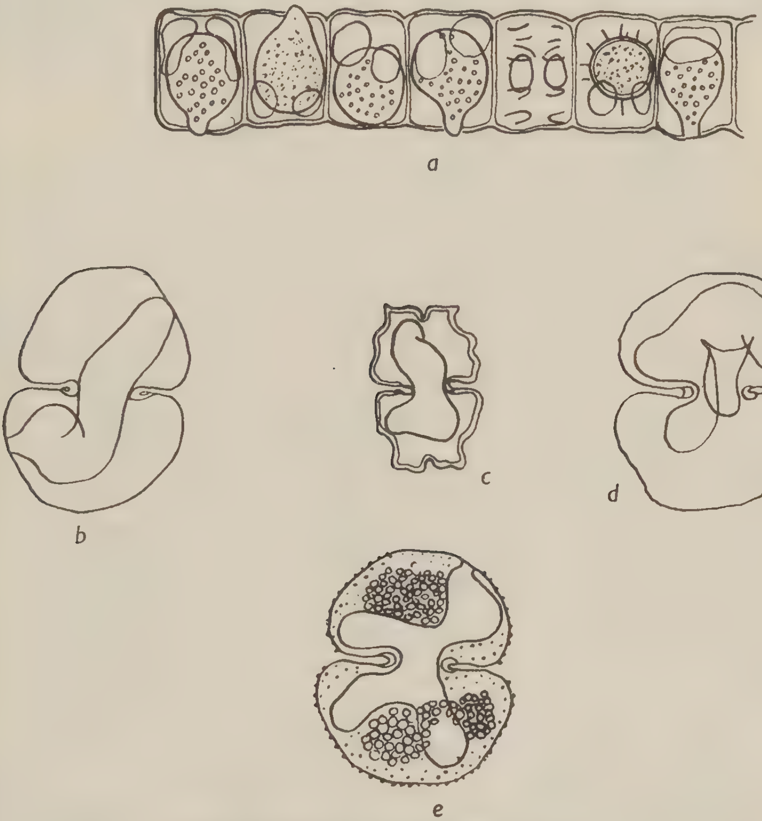
(With Pl. V and 5 Text-figures)

Since *Olpidium hyalothecae* and *O. utriculiforme* were originally described by Scherffel (1926) from Hungary, no further descriptions of them have appeared in the literature. What are considered to be these species were found by me in the bog bordering the northern side of Blelham Tarn near Wray Castle; *O. hyalothecae* occurred in April and May and *O. utriculiforme* in January 1947. *O. hyalothecae* was again found in April on the shore of Esthwaite Water near Hawkshead.

I. *Olpidium hyalothecae* Scherffel

This chytrid is recorded by Scherffel (1926) parasitizing *Hyalotheca mucosa* (Mert.) Ehrenb. and *H. dissiliens* (Sm.) Bréb., but the British material has so far only been found in the latter.

Hyalotheca is usually surrounded by a wide mucilage sheath which was apparently not present in the Hungarian material (Text-fig. 1*a*). When an alga is surrounded by mucilage it is usual for the chytrid zoospore to encyst on the surface of the mucilage and for the germ tube to grow to the surface of the host cell (e.g. *Dangeardia mammillata* Schröder on *Eudorina elegans*, Canter, 1946). In *Olpidium hyalothecae* this is not so; the zoospore itself penetrates the mucilage until it reaches the host cell. A few specimens were seen in which the zoospore had just begun or had penetrated half way through the mucilage (Text-fig. 2*f*, *h*). From the tip of every zoospore settled on the host wall, a faint line can be seen passing to the external surface of the mucilage, either vertically or somewhat diagonally (Text-fig. 2*d*, *f*–*h*). This line indicates the path of the zoospore through the mucilage. The contents of the zoospore pass into the alga while the empty zoospore case and path of penetration remain clearly visible. Very early stages of development of the parasite within the host cell are obscured by the dense contents, and the sporangium is first visible as a spherical, walled body with large scattered oil globules (Text-fig. 2*i*). Before the sporangia are mature the apex of the neck is filled with mucilage which extends for a short distance outside the cell (Text-fig. 2*j*, *k*). The mature sporangia are pear-shaped, 20–9μ high × 13–6·5μ broad, with a short neck which reaches up to the surface of the host wall but never projects beyond. Although the actual moment of dehiscence was never seen, the zoospores appear to be fully formed within the sporangium. They are spherical, $3-4\,\mu$ in diameter with a small posterior oil globule, and a flagellum about $12\,\mu$ long. Owing to the presence of the mucilage sheath around the alga



Text-fig. 1. *a. Olpidium hyalothecae* after Scherffel (1926). *b–e, O. utriculiforme* after Scherffel (1926). × 500.

it is puzzling to know how the zoospores escape into the external medium. Staining with Indian ink shows that the mucilage in the vicinity of the empty sporangia has disappeared (Text-fig. 2*e*), and the Indian ink particles rapidly enter these sporangia. It would seem that the mucilage does not disappear until the sporangium is mature, for several specimens

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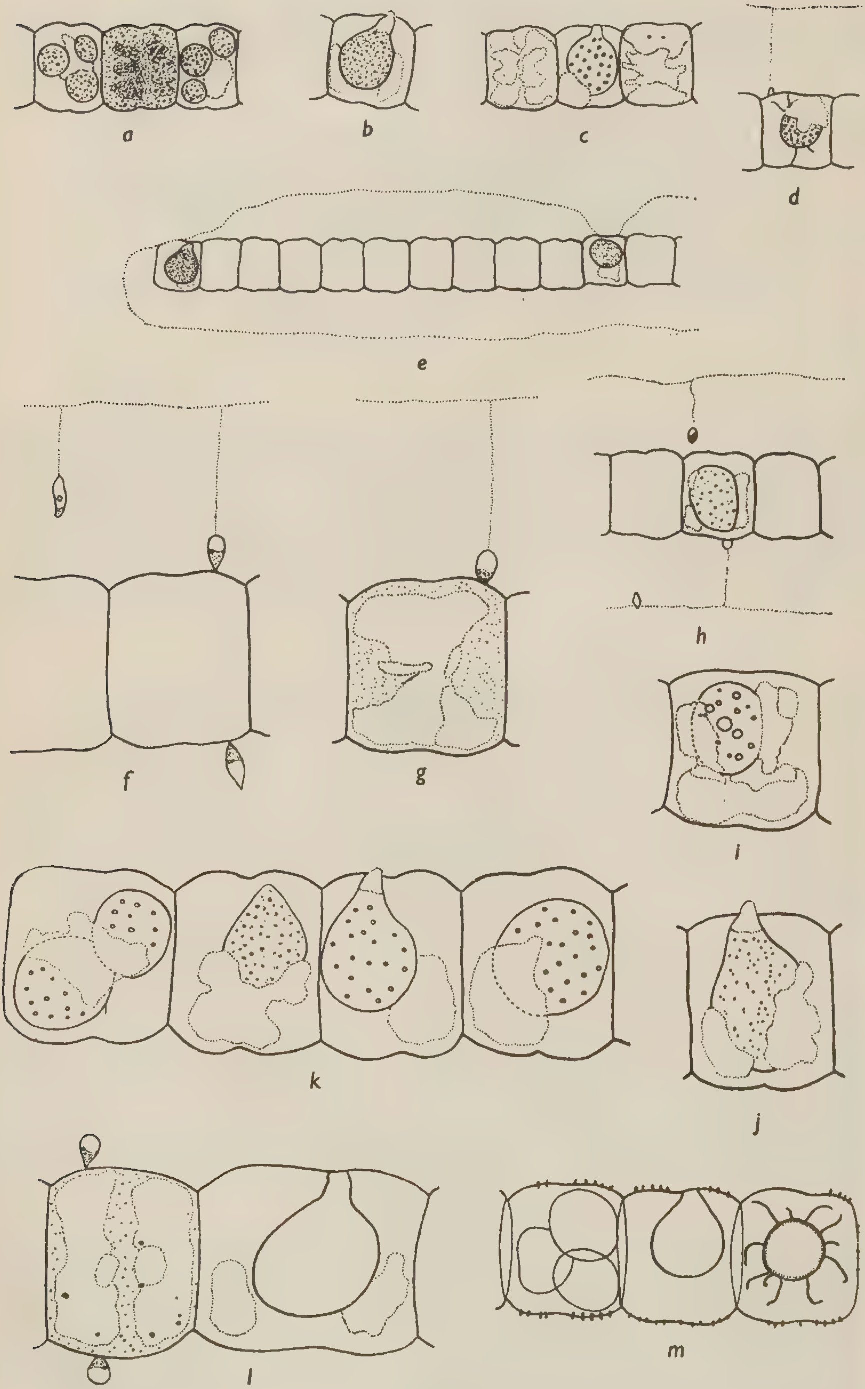
Legend to Text-fig. 2.

Text-fig. 2. *Olpidium hyalothecae*. *a–c*, stages in development of the sporangium. *d*, resting spore. *e*, part of a *Hyalotheca* filament in Indian ink; note absence of mucilage sheath in region of empty sporangia; the latter are filled with Indian ink particles. *f*, *g*, zoospores penetrating the mucilage or recently settled on the host wall; the vertical dotted line indicates their path of penetration. *h*, immature sporangium with empty zoospore case and path of penetration clearly visible. *i*, young sporangium. *j*, immature sporangium mucilage papilla well developed. *k*, *l*, immature, mature and empty sporangia. *m*, empty sporangia and resting spore after staining with Chlorazol black E in lactophenol. *a–d*, *h*, × 500; *f*, *g*, *i–l*, × 1050; *e*, × 300; *m*, × 800.



Text-fig. 2.

were observed in which, although the oil globules of the zoospores were delimited, no mucilage had been dissolved.

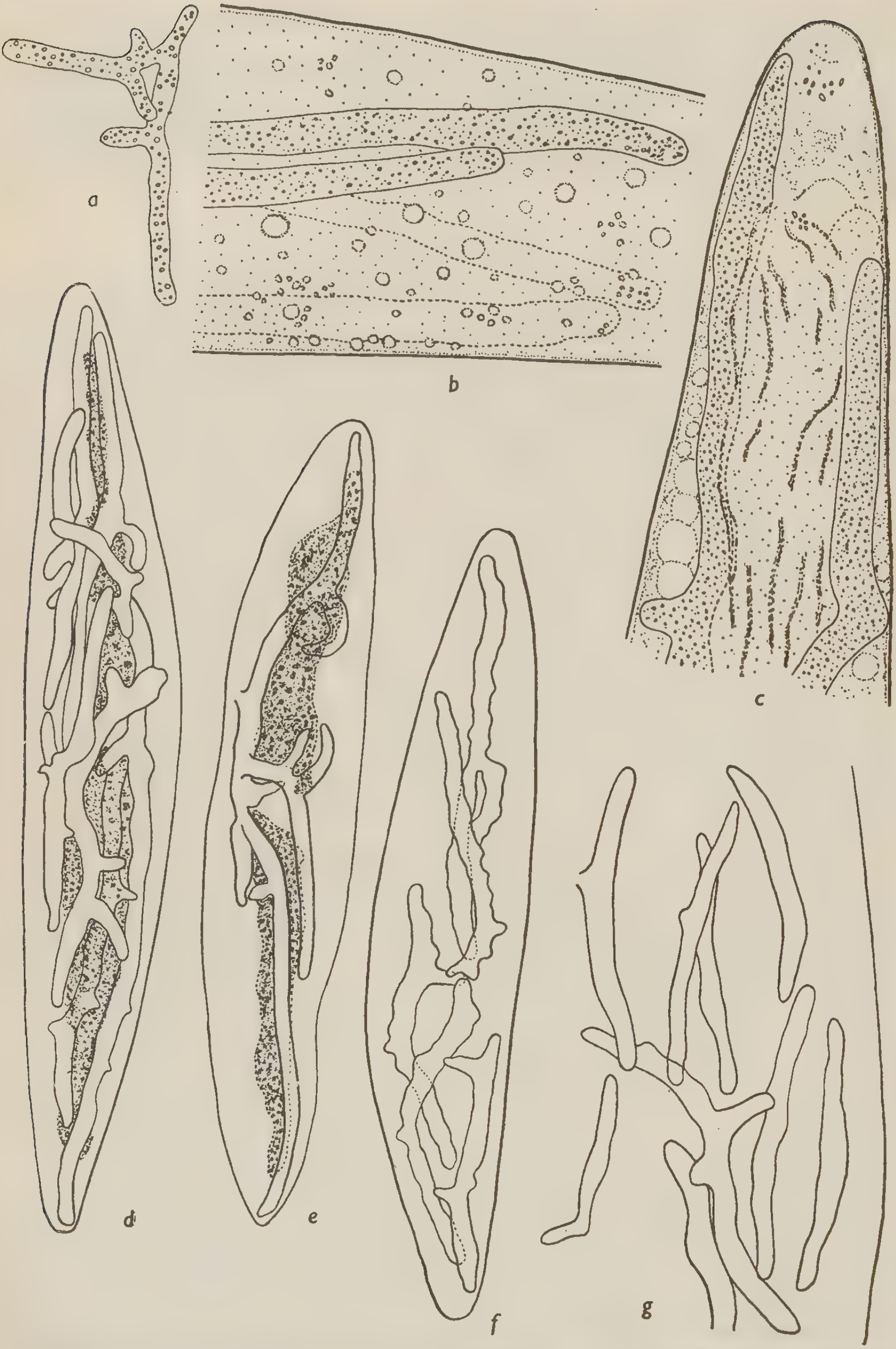
Resting spores were rare, but the few seen agree with those described by Scherffel. They are spherical, 10 μ in diameter, with a smooth wall, the outer surface of which bears a few long processes (Text-fig. 2*m*) which are clearly visible after staining with Chlorazol black E. Their germination was not observed.

II. *OLPIDIUM UTRICULIFORME* SCHERFFEL

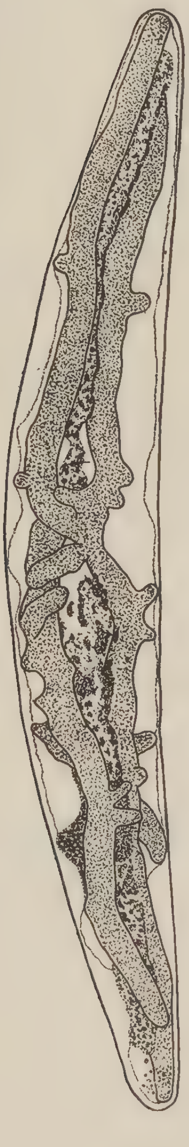
This chytrid (Text-figs. 3–5 and Pl. V, figs. 1–4) occurred as a parasite in *Closterium lunula* Ehrenb., *C. costatum* Corda and *C. dianeae* Ehrenb. Owing to the dense algal contents very young stages in infection were not observed. It is thus unknown whether the whole content of the zoospore passes into the host as a naked mass of protoplasm which soon becomes walled, or whether the zoospore puts forth a germ tube with a delicate wall which subsequently thickens.

The thallus, when first distinguishable, is surrounded by a thin wall and consists of a narrow unbranched tube with hyaline cytoplasm containing large oil globules (Text-fig. 3*a*). This tube elongates, branches, the wall thickens and at maturity the whole forms a single sporangium. The mature thallus is often extensive, consisting of several tubes (up to 550 μ long by 11–20 μ wide) which taper slightly towards their extremities and run parallel to the length of the *Closterium* (Text-fig. 4). Shorter lateral branches are also present. As well as these extensive thalli, small unbranched or little branched forms occur (50–90 μ long by 5–8 μ wide, Text-fig. 3*f*, *g*). The number of parasites in a host cell varies from one to twelve and the smaller tend to be formed in cells which already contain a large thallus or where several are crowded together.

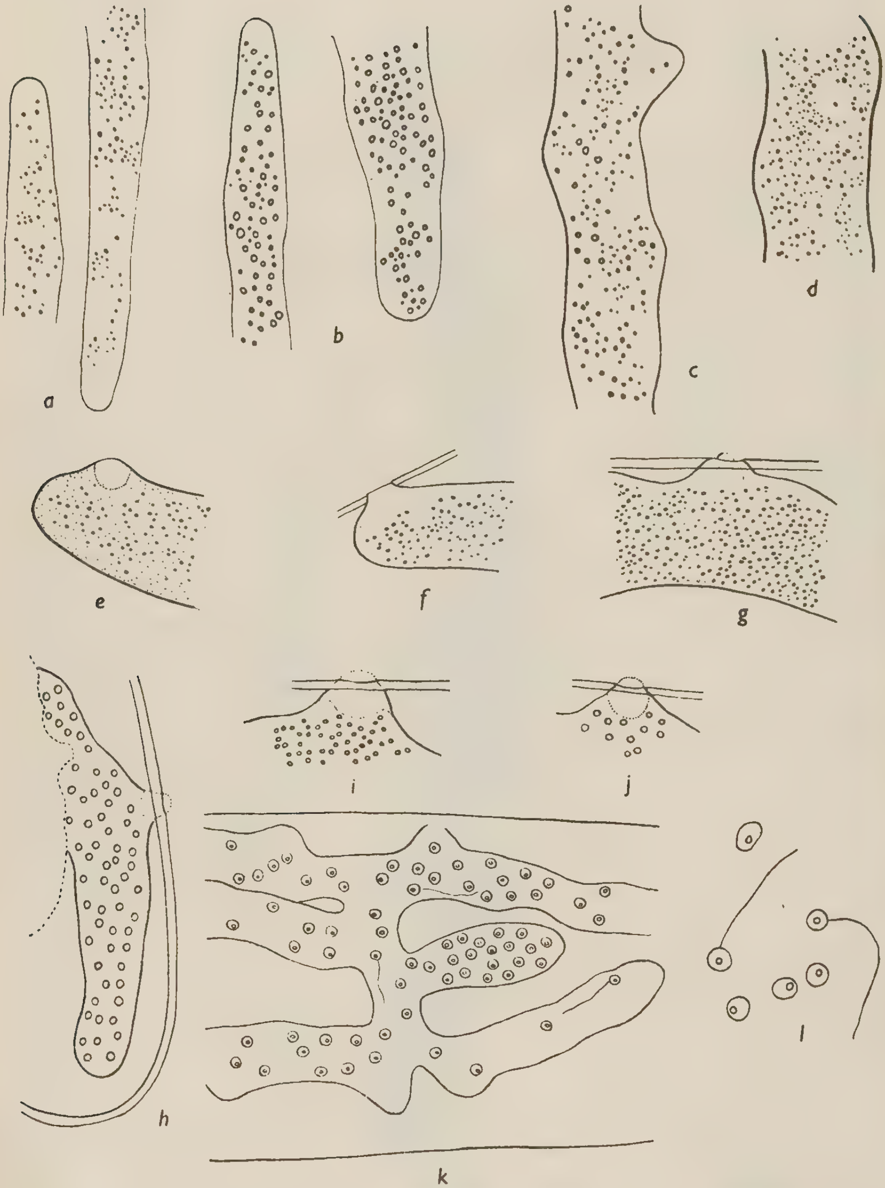
As the parasite grows the host content shrinks, the protoplasm becomes foamy and vacuolate and the periphery is occupied by minute streaming granules. By the time the chytrid is mature the two algal chloroplasts are reduced to brown masses. Young thalli, when stained with chlor-zinc-iodide, give no reaction, while dehisced sporangia stain faintly purple. The changes in the protoplasm during the development of this fungus are similar to those recorded for the majority of chytrids. At first the fungal content is hyaline with oil globules collected together in certain regions (Text-fig. 5*a*, *b*). These become smaller and more evenly distributed (Text-fig. 5*d*). Later the protoplasm is granular, with scattered, highly refractive granules (Text-fig. 5*e*) which increase in size to form the oil globules of the zoospores. Before the zoospores are mature certain areas of the thallus adjacent to the *Closterium* wall become gelatinous forming a dully refractive plug which projects slightly beyond the external surface (Text-fig. 5*g*–*j*). The hundreds of zoospores fully formed within the sporangium are liberated either into the external medium or into the cavity of the host cell. They are spherical, 2·4–2·8 μ in diameter, with a conspicuous oil globule, somewhat lateral in position, and a posterior flagellum 14–19 μ long; they may be amoeboid or exhibit a smooth gliding movement.



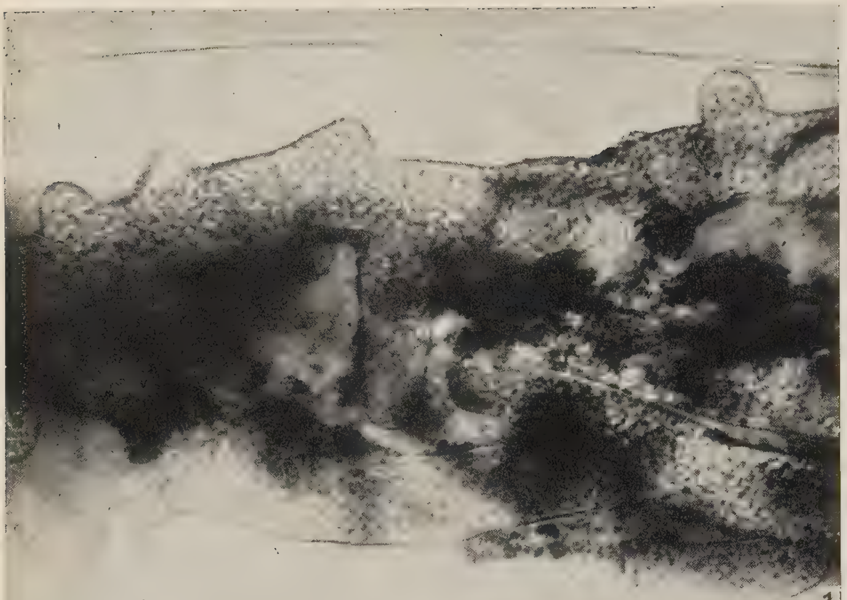
Text-fig. 3. *Olpidium utriculiforme*. *a*, very young thallus. *b*, *c*, young sporangia with branches extending along the length of the *Closterium* whose content in (*c*) shows signs of disintegration. *d–f*, host cells containing from one to several sporangia. *g*, small unbranched or little branched sporangia. *a–c*, *g*, ×500; *d–f*, ×185.



Text-fig. 4. *Olpidium utriculiforme.* An extensive sporangium in *Closterium lunula* Ehrenb. ×220.



Text-fig. 5. *Olpidium utriculiforme*. *a–h*, protoplasmic changes in the sporangium up to the formation of the oil globules of the zoospores; in *e–j* dehiscence papillae are visible. *k*, part of a sporangium with mature zoospores. *l*, zoospores. *k*, × 500; the rest, × 1050.



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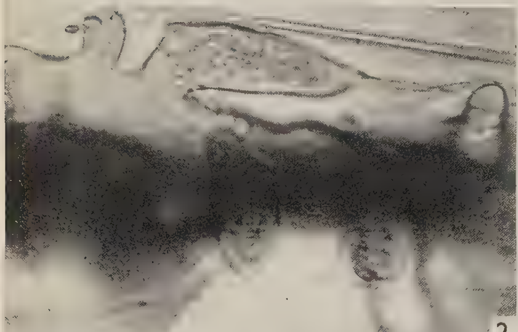

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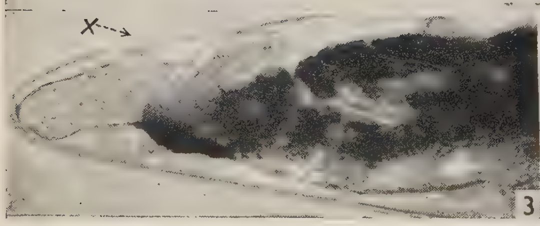

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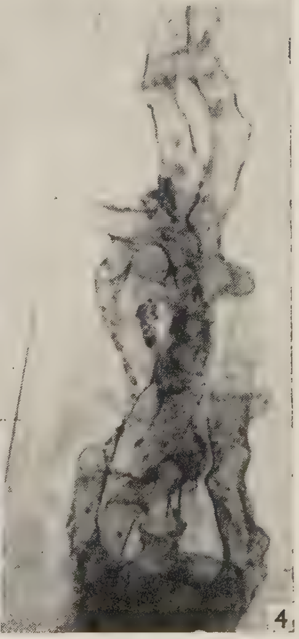

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The material of *Olpidium utriculiforme* was extensively attacked by a filamentous fungus (possibly a species of *Pythium*) and disappeared without the formation of resting spores.

Scherffel's figures of *Olpidium utriculiforme* are shown in Text-fig. 1 *b–e*. The form described above agrees in most details with Scherffel's organism, except for the presence of the extensive thalli. The size and degree of branching of the fungus is clearly limited by the host size and whereas Scherffel's form occurred in the smaller desmids such as *Cosmarium botrytis* and *Euastrum* sp., mine was found in large species of *Closterium*. A similar condition exists in *Myzocytium megastomum* De Wildeman which attacks desmids, where small reduced thalli develop in the smaller algae (Canter, 1947).

Again, *Olpidium utriculiforme* shows a striking resemblance to *Mitochytidium ramosum* Dangeard (1911), but in this form the thallus bears rhizoids. This raises the question to what extent the presence or absence of rhizoids should be used to determine generic distinction. In *Chytidium lagenaria* Schenk pro parte within the same collection I have found thalli with the apophysis bearing an extensive rhizoidal system, whereas in others it is absent. This was also recorded very rarely by Sparrow (1936). It is only when the resting spores of *Mitochytidium* are more definitely known and those of *Olpidium utriculiforme* discovered that the true affinities of these fungi will become clear.

SUMMARY

Olpidium hyalothecae Scherffel and *O. utriculiforme* Scherffel are recorded for the first time in Great Britain.

My thanks are due to the Director of the Freshwater Biological Association for the use of a laboratory in which this work was carried out and especially to Prof. C. T. Ingold for reading the manuscript.

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EXPLANATION OF PLATE V

Olpidium utriculiforme Scherffel

Fig. 1. Part of a mature sporangium. ×750.
Fig. 2. Immature sporangium. ×750.
Fig. 3. Immature sporangium with dehiscence papilla visible at *X*. ×400.
Fig. 4. Empty sporangia. ×360.

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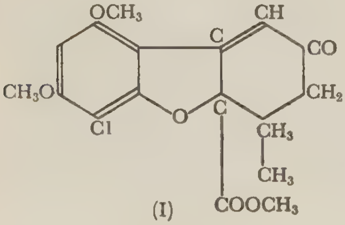
A SUBSTANCE CAUSING ABNORMAL DEVELOPMENT OF FUNGAL HYPHAE PRODUCED BY *PENICILLIUM JANCZEWSKII* ZAL.

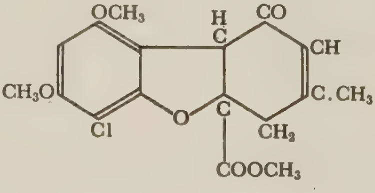
III. IDENTITY OF 'CURLING FACTOR' WITH GRISEOFULVIN

By P. W. BRIAN, P. J. CURTIS and H. G. HEMMING
Butterwick Research Laboratories, The Frythe, Welwyn, Herts

In a previous communication (Brian, Curtis & Hemming, 1946) a metabolic product of *Penicillium janczewskii* Zal. was described, which had the unusual property of causing marked morphological abnormalities in the development of *Botrytis* hyphae. This substance, known as 'curling factor', was at first thought to contain only carbon, hydrogen and oxygen and the molecular formula $C_{20}H_{20}O_9$ was proposed (McGowan, 1946). Subsequent investigation showed that it also contained chlorine (Grove & McGowan, 1947) and was, in fact, chemically identical with griseofulvin ($C_{17}H_{17}O_6Cl$), first isolated by Oxford, Raistrick & Simonart (1939) from the mycelium of *Penicillium griseofulvum* Dierckx. The structural formulae proposed by Oxford *et al.* (I) and by Grove and McGowan (II) are shown below:



(I)



(II)

The present communication is intended to give the biological evidence for the identity of griseofulvin and 'curling factor' and to present certain other data concerning its production that have arisen from the discovery of this identity.

BIOLOGICAL CONFIRMATION OF IDENTITY OF 'CURLING FACTOR' WITH GRISEOFULVIN

Solutions (100 μg./ml.) of authentic griseofulvin from *P. griseofulvum*, kindly supplied by Prof. Raistrick, and of 'curling factor' from *P. janczewskii* were made up in Weindling solution. These were assayed by a method previously described (Brian *et al.* 1946), *Botrytis allii* being the test organism. Both samples produced the typically stunted and distorted growth and were of equal activity. A further sample of griseofulvin, also supplied by Prof.

Raistrick, which had been obtained from *Penicillium patulum* (Raistrick, 1947) was also assayed, with similar results. These observations, taken in conjunction with the chemical evidence already quoted, establish beyond doubt the identity of 'curling factor' and griseofulvin. Thus, on the evidence at present available, it appears that griseofulvin is produced by at least three different species belonging to three distinct major divisions of the genus *Penicillium*: *P. griseofulvum* Dierckx (Assymetrica-Funiculosa), *P. janczewskii* Zal. (Lanata-Divaricata), *P. patulum* Bain. (Fasciculata).

ACTIVITY OF CULTURE FILTRATES OF *PENICILLIUM JANCZEWSKII* AND *PENICILLIUM GRISEOFULVUM*

Three strains of *P. griseofulvum* and two strains of *P. janczewskii* were grown on a Czapek-Dox medium, containing 7·5 % of crude glucose, in 100 ml. flasks and samples were taken periodically for assay. The strains used were as follows:

No. 29. *P. janczewskii*: first obtained in September 1942 as a laboratory contaminant (i.e. before our first isolations from Wareham soil).
No. 384. *P. janczewskii*: isolated from a cave soil (Read's cavern, Somerset) in 1947.
No. 374. *P. griseofulvum*: obtained from Prof. Raistrick (LSHTM Catalogue No. P 38)—the strain used for the original isolation of griseofulvin.
No. 375. *P. griseofulvum*: obtained from Prof. Raistrick (LSHTM Catalogue No. P 68).
No. 370. *P. griseofulvum*: obtained from Centraalbureau voor Schimmelcultures, Baarn, Holland.

The results of the experiment are shown in Table 1. Both 'curling activity' and inhibition of germination are recorded. In the code used for indicating 'curling activity' the index figure following the letter S denotes the greatest dilution producing marked stunting of the germ-tube and the index figure following the letter C the greatest dilution producing any morphogenetic effect. The figures given under the heading 'inhibition-assay' indicate the greatest dilution completely inhibiting germination.

Table 1. *Assays of culture filtrates from* Penicillium griseofulvum *and* Penicillium janczewskii

Culture No.	Species	'Curling' assay (days growth)				Inhibition assay (days growth)			
		5	9	13	17	5	9	13	17
29	*Janczewskii*	$S_{16}C_{128}$	$S_{32}C_{256}$	$S_{64}C_{512}$	$S_{32}C_{256}$	—	—	—	—
384	*Janczewskii*	$S_{8}C_{256}$	$S_{8}C_{128}$	$S_{16}C_{512}$	$S_{16}C_{512}$	—	—	—	—
374	*Griseofulvum*	$S_{8}C_{64}$	$S_{64}C_{1024}$	$S_{32}C_{1024}$	$S_{128}C_{1024}$	—	—	—	—
375	*Griseofulvum*	$S_{0}C_{0}$	$S_{64}C_{0}$	$S_{64}C_{0}$	$S_{64}C_{0}$	6	24	16	16
370	*Griseofulvum*	$S_{0}C_{0}$	$S_{0}C_{0}$	$S_{2}C_{0}$	$S_{4}C_{64}$	—	—	—	—

Both strains of *P. janczewskii* developed typical 'curling activity'. This now appears to be quite characteristic of this species as all strains examined, from three different sources, have shown the activity. One strain of *P. griseofulvum* (No. 374—Raistrick P. 38) showed similar, possibly higher, activity. The Baarn strain of *P. griseofulvum* showed a slight activity, developed very late. The remaining strain of *P. griseofulvum* (No. 375—Raistrick P 68), though morphologically very similar to No. 374, showed no 'curling activity' but did appear to produce a fungistatic substance, preventing germination of *Botrytis allii*.

GRISEOFULVIN CONTENTS OF MYCELIUM AND CULTURE FILTRATE

Oxford *et al.* (1939) isolated griseofulvin from the mycelium of *Penicillium griseofulvum*. From 3000 g. of mycelium they obtained 50 g. of griseofulvin. To obtain the 3000 g. of mycelium, 259 l. of medium were used, the cultures being incubated for 65–85 days at 30° C. We have extracted 'curling factor' from 11 to 14-day culture filtrates of *P. janczewskii* obtaining yields of 150 mg./l. or about 40 g. from 259 l. It thus appeared probable that by extracting the mycelium as well as the culture filtrate of *P. janczewskii* yields might be approximately doubled. This has in fact been found to be the case as illustrated by the following data for a batch of fifty cultures each on a litre of medium:

Volume of culture filtrate: 50 l.
Weight of griseofulvin extracted from culture filtrate: 8·5 g.
Dry weight of mycelium: 850 g.
Weight of griseofulvin extracted from mycelium: 12·0 g.

RELATION OF CHLORIDE CONTENT OF MEDIUM TO PRODUCTION OF GRISEOFULVIN

Chlorine is not generally considered essential for the growth of fungi, but it is clearly essential for the production of griseofulvin. Allowing for the probable inefficiency of the methods of extraction used it seems likely that the real yield of griseofulvin per litre of culture (in both mycelium and filtrate) is of the order of 500 g. This would require that the fungus be supplied with 50 mg. chlorine per litre or approximately 0·01 % KCl. A concentration of KCl of this order is necessary to obtain maximum titres in cultures of *P. janczewskii* as will be seen by reference to the experimental data in Table 2. The fact that assays were obtained in previous experiments (Brian *et al.* 1946) on media with no added chloride (e.g. medium *N*) can be accounted for by impurities in the dextrose used and HCl used for adjusting the initial pH. The composition of medium *N* is as follows: potassium nitrate, 2·3 g.; potassium dihydrogen phosphate, 1·0 g.; hydrated

Table 2. *Relation between chloride content of medium N and production of griseofulvin by* Penicillium janczewskii

Potassium chloride (%)	Assay (days) 4	6	8	10
—	$S_2 C_{32}$	$S_{16} C_{256}$	$S_8\ C_{256}$	$S_8\ C_{256}$
0·001	$S_4 C_{64}$	$S_{32} C_{1024}$	$S_{64}\ C_{2048}$	$S_{128} C_{1024}$
0·005	$S_2 C_{64}$	$S_{32} C_{1024}$	$S_{64}\ C_{4096}$	$S_{256} C_{8192}$
0·01	$S_8 C_{128}$	$S_{32} C_{1024}$	$S_{128} C_{4096}$	$S_{128} C_{8192}$
0·1	$S_2 C_{64}$	$S_{32} C_{1024}$	$C_{128} C_{4096}$	$S_{256} C_{8192}$
1·0	$S_4 C_{128}$	$S_{64} C_{1024}$	$S_{64}\ C_{4096}$	$S_{128} C_{4096}$

magnesium sulphate, 0·5 g.; dextrose, 75·0 g.; minor element concentrate, 1 ml.; distilled water, 1000 ml. In this experiment (Table 2) all constituents were of the purest grade available and pH was not adjusted but, nevertheless, there was apparently sufficient chloride present to result in the development of appreciable 'curling activity'. The highest concentration of potassium chloride (1·0 %) somewhat reduced the vigour of growth of the mould.

SUMMARY

The 'curling factor' produced by *Penicillium janczewskii* has been shown to be identical with the substance known as griseofulvin ($C_{17}H_{17}O_6Cl$) previously isolated from the mycelium of *P. griseofulvum* and *P. patulum*. All strains of *P. janczewskii* examined, from different sources, have shown the capacity to produce griseofulvin, but not all strains of *P. griseofulvum* have done so.

Yields of griseofulvin from *P. janczewskii* can be approximately doubled by extracting the mycelium as well as the culture filtrate; by this means yields of the order of 400 mg. per litre of culture medium have been obtained. A chlorine content of the medium corresponding to 0·005–0·01 % potassium chloride is optimum for griseofulvin production.

We are much indebted to Prof. H. Raistrick, F.R.S., for the provision of authentic samples of griseofulvin and cultures of *P. griseofulvum*.

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STUDIES ON SOME DISEASES OF SAINFOIN (*ONOBRYCHIS SATIVA*)

II. THE LIFE HISTORY OF *RAMULARIA ONOBRYCHIDIS* ALLESCHER

By S. J. HUGHES

(With Plate VI and 22 Text-figures)

Ramularia onobrychidis Allescher causes a leaf spot of sainfoin. Attacked leaflets are killed and fall away readily from the midrib and in a thick crop the disease can cause quite a heavy loss of leaflets. In Great Britain it has been recognized only in the Vale of Glamorgan, South Wales. It was found there first in December 1943 on the local strain of common sainfoin and later also on the Hampshire and Cotswold commercial strains.

Historical review

The fungus was first described by Allescher (1891) as follows: '*R. Onobrychidis* Allescher nov.spec. Maculis circularibus, brunneis; caespitulis hypophyllis; conidiis cylindraceis, rectis vel leniter curvatis, utrinque obtusiusculis, plerumque 1 septatis, hyalinis, *ca.* 20–30 × 3–5. Hab. in foliis vivis Onobrychidis sativae in campis prope Pasing juxta München Bavariae superioris; leg. Allescher.'

Prillieux and Delacroix (1893) used the same name to describe a *Ramularia* on sainfoin thus: '*Ramularia Onobrychidis*, nov.sp., parasite sur les feuilles de Sainfoin (*Onobrychis sativa*).

Le *Ramularia* qui accompagnait de temps en temps ces périthèces d'*Ascochyta* (*A. Orobi* var. *Onobrychidis* Prill. & Delacr.) se présente comme de très petites taches, blanches, pruineuses; ses sporophores simples, septés, d'environ 50 × 3 μ, se terminent par une courte chaîne de conidies de 15–30 × 4·5–5 μ, plus ou moins atténuées ou arrondies au deux extrémités, continues au debut et qui acquierent deux ou trois cloisons à la maturité.

Cette mucedinée nous paraît être une form conidienne de l'*Ascochyta* dont nous venons de parler.'

Saccardo (1895), when compiling *Ramularia onobrychidis* Allesch., treated *R. onobrychidis* Prill. & Delacr. as a synonym; he reproduced both diagnoses. Although Allescher did not mention the conidiophores, it is not to be doubted that the same species was described by Prillieux and Delacroix.

No new observations have been made on the fungus since 1893, although it has been compiled by various authors with the use of the original descriptions. The fungus has been recorded in various countries: in Denmark by Rostrup (1902), in South Bavaria (Innsbruck) by Magnus (1905), North Italy by Ferraris (1913) and in Serbia by Ranojevic (1914). *R. onobrychidis* is also compiled by Massee (1915) and Jaczewski (1917), but these are doubtful records for Great Britain and Russia respectively. Stevenson (1926) recorded *R. onobrychidis* All. on *Onobrychidis sativa* and *O. viciaefolia* in Russia, Germany, Sweden, Yugoslavia, France and Denmark. Sainfoin is also grown in British Columbia, Bulgaria, England, Finland, Holland, Switzerland and Hungary.

Description of spots

Only a single field has so far provided an abundance of leaf spots throughout the crop, but elsewhere, uncut headlands, where the air is kept moist by tall growth of sainfoin and of grasses, have provided further heavy centres of infection.

The diseased spots are very variable (Pl. VI, fig. 1). When young they are dark brown, but as they increase in size they grow paler from the centre outwards and either produce a ring spot or a uniformly pale coloured one. They are always darker on the adaxial side than on the abaxial side of a leaflet and even when a ring spot is seen on the upper side only a fawn area will be observed below. The dark colour is due to a darkening of the cell walls of the leaflet and in part to brown substances developed or deposited in some of the cells, especially certain palisade cells which are much larger than the adjacent ones.

Sporulation is restricted to dead tissues and occurs all the year round, but is most active in late spring, summer, and early autumn. Under suitable conditions numerous sporodochia are formed and their conidial chains cover both sides of the spot with a white meal. With the approach of winter the production of fresh sporodochia is superseded by the production of sclerotia.

In old spots sclerotia form a well-marked greyish ring (Pl. VI, fig. 3) and when waterlogged the individual sclerotia can be seen even with the naked eye as a circle of minute black dots enclosing the effete remains of sporodochia and of their conidiophores.

In younger spots, which have not produced sporodochia, sclerotia are aggregated towards the centre. Under moist conditions sclerotia may develop in fallen leaflets on the ground. Infected leaflets fall readily on being disturbed, or they may dry out while still attached to the midrib. They are readily blown about in the wind and this, no doubt, helps to spread infection to other parts of the same field and perhaps to neighbouring fields.

In summer the conidiophores form a circular area on the spot, but in winter the conidiophores develop from sclerotia and not from sporodochia, and, as might be expected, they are found in a mealy ring. In spots with a central group of sclerotia the conidiophores are central as in summer spots.

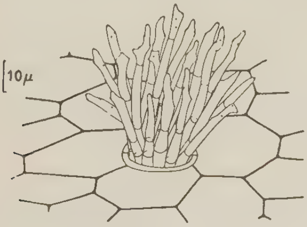
Method of infection and development of mycelium

Examination of leaflets, inoculated by applying drops of spore suspension to their surfaces, showed that both stomatal and cuticular penetration occurs. In mounts stained with cotton blue some germ tubes were seen to pass over a stoma and pierce the cuticle some distance away, whilst others penetrated the stomatal pore. In the latter, the germ tube swelled in the substomatal cavity and then branched out into the surrounding tissue.

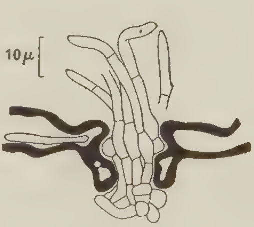
Death of the leaflet cells results in their collapse, but cells of the epidermal layers are often very readily distinguished even in old spots, presumably because of their sturdier structure. Fungal hyphae are found in all parts, but the mycelium is denser immediately beneath the epidermal layers. Hyphae are mostly intercellular, but they are also commonly found inside epidermal cells where, however, they have not been seen to develop any further. Mycelium has been seen in the phloem, but not in the xylem of the vascular bundles, whereas hyphae of *Pleospora herbarum* were encountered abundantly in both xylem and phloem elements of leaflets infected by this fungus (Hughes, 1945). The mycelium is hyaline throughout the leaflet, but as winter approaches the walls darken slightly and more so during the development of sclerotia.

Development of sporodochia

In *Ramularia vallisumbrosae* the sporogenous system is subcuticular (Gregory, 1939); in *R. onobrychidis* it is entirely subepidermal and substomatal. Sporodochia are restricted to the substomatal cavities and as mentioned previously, are found sporulating actively in late spring, summer and early autumn. Their tufts of conidiophores protrude through the dilated stomatal pores (Text-fig. 1) between the crushed guard cells, but in no case has any rupture of the epidermal tissues been seen.



Text-fig. 1.



Text-fig. 2.

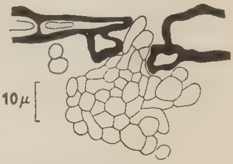
Text-fig. 1. Sporodochial conidiophores protruding through a dilated stomatal aperture. The conidial chains have fallen away, but the scars are conspicuous. Cardiff, 11 August 1944.

Text-fig. 2. Transverse section of young sporodochium developing between and below the guard cells of the upper epidermis. Cardiff, 11 August 1944.

The sporodochium begins as a small knot of hyphae in the substomatal cavity, but whether this arises from one or more than one hyphal branch has not been determined. From this knot, a fascicle of hyphae, which become conidiophores, soon pushes up through the stoma; characteristic chains of spores are then developed (Text-fig. 4). The sporodochium enlarges by the division of its cells, and not uncommonly the regular outward elongation of some of the cells towards the stoma to produce conidiophores gives the sporodochium the appearance of a meristematic tissue (Text-fig. 2). Such a regular arrangement of cells in substomatal sporodochia was observed by Killian (1926) as opposed to the more irregular arrangement when sporodochia arise elsewhere. He stated: 'Les différences structurales des ébauches se manifestent encore à l'état adulte: Les conidiophores interstomataires sont formés de filaments parallèles, les autres se présentent sous forme de pelotons globuleux.' This parallel arrangement of which he spoke is very characteristic of the 'necks' of sclerotia.

Protrusion of conidiophores through the stomata appears, according to Killian (1923, 1926) and Gregory (1939), to be less common than their rupturing of the epidermal layer.

In winter old sporodochia sometimes turn pale brown, but then seldom produce a fresh crop of spores when placed in a moist atmosphere. Killian (1926) found that sporodochia of *Ramularia parietaria* Passer. (a species which does not produce sclerotia) turned brown, especially at their bases, but retained their ability to produce conidia after a period of rest.



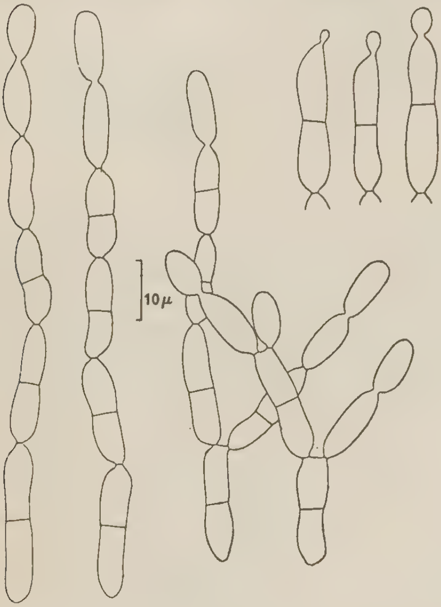
Text-fig. 3. Transverse section of young sporodochium which has not yet produced conidiophores. Upper epidermis. Cardiff, 11 August 1944.

CONIDIOPHORES AND CONIDIA

The conidiophores of the sporodochia are unbranched, filiform, hyaline, two to three or more septate with the basal cell usually swollen; they are narrower than the conidia and measure 30–50μ long and 2–3·5μ wide. Each conidial chain, after dispersal, leaves a scar on the conidiophore. As many as seven scars have been observed on a single conidiophore, but restricted to its terminal cell. The conidiophore may be geniculate or faceted, according to the position of the scar, but is usually faceted (Text-fig. 1). In young sporodochia or recently sprouted sclerotia the condiophores bear one terminal facet, but as they become older and further spore chains develop, the position of each is clearly seen as a more refractive circle of wall substance.

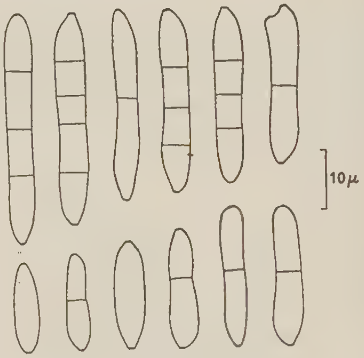
The hyaline conidia vary considerably in length (7–42μ) and vary in form from phragmospores through didymospores to amerospores. The first conidium arises as a globose swelling at the apex of a conidiophore; it increases in length, becomes oval, and eventually almost cylindrical with a slightly tapering base. A septum separates the conidium from the conidiophore and the conidium may or may not become variously septate. The first spore is not detached from the conidiophore, but buds off another spore at its apex which becomes slightly tapering. By such successive acropetal growth a chain of dry conidia is produced. It is evident then that only the first-formed conidium is ever in actual contact with the conidiophore that bears it but: 'The first-formed spores, which are at the base of the chain, first act as part of the thallus, in that they support in the air, and carry food to, the later formed spores. "Leur séparation, qui les rend aptes à multiplier et à disperser le Champignon à la façon des spores, n'arrête pas nécessairement leur activité végétative".' (Mason, 1937.) Catenulation has been observed in all lesions and the chains may be up tc seven conidia in length and quite probably longer. It is quite common to find a conidium, not necessarily the oldest, or first formed, budding off two conidia at its apex and thereby initiating a branched chain. No conidium has been seen to develop from an old scar so that the conidia are *terminus thallospores*; the production of spore chains, therefore, increases possibly tenfold the number of units presented for dissemination.

A successive reduction in the length of the conidia, as they are successively more distant from the conidiophore is evident, and in addition, the number of septa to each spore is also reduced (Text-fig. 4). Whereas the first-formed conidium is a phragmospore or didymospore, the distal conidia are invariably amerospores; some of the longer unseptate conidia may well be potential didymospores. The production of successively smaller conidia with fewer septa is to be expected. Bliss and Fawcett (1944) have found that a similar decrease in spore length takes place in the spore chains of *Alternaria citri* Ellis & Pierce and state: 'This is perhaps due to the relative availability of food at different points along the spore chain. In this acropetal type of sporulation, the first or oldest spore grows directly from the conidiophore; but the second spore, at the apex of the first, must obtain its food by translocation through cells of the first spore. Similarly, food for succeeding spores must pass through all the older spores in the chain, a process that naturally becomes more difficult at each step....A similar effect on spore size may also result from the branching of the spore chain. Smaller spores develop after the food stream has been divided.' This may well be the explanation for the similar phenomenon found in this *Ramularia*. When diseased leaflets with sporulating spots are tapped over a dry slide,



Text-fig. 4.

Text-fig. 5.



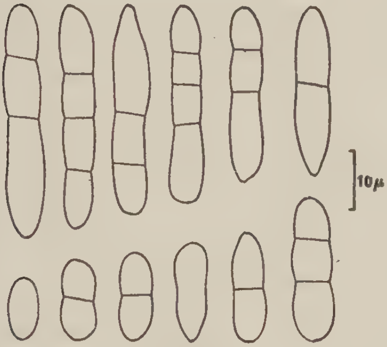
Text-fig. 6.

Text-fig. 4. Branched and unbranched chains of conidia deposited on a glass slide by gently tapping a fruiting lesion over it. The gradual and successive reduction of conidial length in each chain is quite evident. Southerndown, 18 March 1944.

Text-fig. 5. Development of conidia by apical budding. Gileston, 18 March 1944.

Text-fig. 6. Conidia from leafspots on sainfoin collected from Experimental Plot, Ely, Cardiff, during a very dry spell. 11 August 1944. The conidia are conspicuously narrower than those of Text-figs. 7–10.

conidia or chains of conidia readily become detached from the conidiophores; by covering the slide carefully with a dry cover-slip the conidial deposit can be examined. The dissemination of conidia by an air current can be observed under a binocular microscope when a jet of air is allowed to impinge on a fruiting leaf spot. The conidia are *dry blastospores* (Mason, 1937) and in the field are presumably dispersed by wind.



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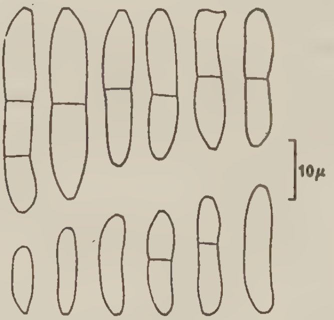

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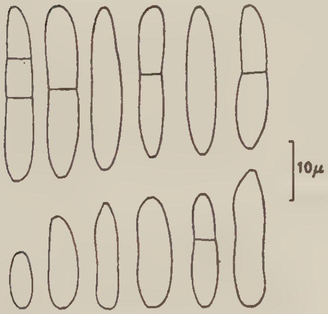

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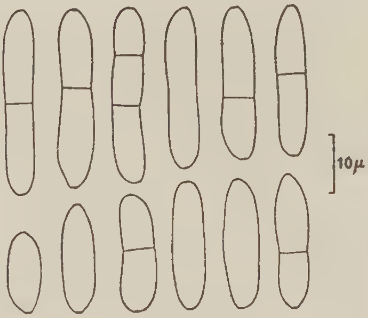


Text-fig. 10.

Text-fig. 7. Conidia from leafspots on sainfoin collected from dry situation. Southerndown, 18 March 1944.

Text-fig. 8. Conidia from leafspots on sainfoin collected from moist situation. Southerndown, 29 October 1944.

Text-fig. 9. Conidia from sclerotia on leaflets of sainfoin collected in fairly moist situation. Southerndown, 9 February 1945.

Text-fig. 10. Conidia from a new infection of sainfoin from fairly dry situation. Southerndown, 21 March 1945.

The spore types vary considerably in measurements and the extreme ranges from all my collections are as follows: phragmospores, 23–42 μ long; didymospores, 12–34 μ long; amerospores, 7–22 μ long.

The spores vary between 2·7 and 6·3 μ wide and the mean from all collections is 4·7 μ.

OCCURRENCE OF THE DIFFERENT FORMS OF CONIDIA ON LEAF SPOTS

Phragmospores, didymospores and amerospores are frequently found together in the same spot, and the three spore types can be produced in the same chain. It was evident that the preponderating type of conidium was closely related to the humidity of the air at the time of conidial development. Gregory (1939) has already directed attention to the fact that *Ramularia vallisumbrosae* displays an even greater range of variation, corresponding with the form genera *Ovularia*, *Ramularia* and *Cercosporella*, and he holds that 'circumstantial evidence points to humidity as one of the controlling factors'. It has already been pointed out that *Ramularia onobrychidis* is favoured by moist conditions and all but about ten of the twenty-four collections of this fungus have been made from damp situations. Again, Gregory has shown how great changes in the type of spore form, produced on a spot, are encountered when leaves are collected in tins where the atmosphere becomes moist. In my collections I took the precaution of allowing all the specimens to dry in air on trays immediately after collecting; presumably under such conditions, spore formation ceased and the spots represented more or less true field conditions.

Conidia were removed from a few spots the day after collecting, mounted in water, and fifty were measured. The type of conidium found present in preponderance was noted and the details of the collections are given in Table 1.

It is clear from Table 1 that a dry atmosphere tends to favour the production of longer conidia with more septa, whilst moist air promotes the development of shorter spores with fewer septa. There is no indication of a seasonal periodicity in the spore type encountered throughout a whole year except that in winter conditions are usually wet and the conidia, thereby, smaller as a result.

To illustrate the effect of humidity on the type of conidium formed, fruiting lesions of ten leaflets of six separate field collections were brushed lightly with a fine camel-hair brush and blown upon to remove all conidia; the leaflets were then placed in a moist chamber for forty-eight hours. Conidia were removed from the lesions at the end of this time, mounted in water and measured. Degree of septation and range in length with a mean of fifty measurements are given in Table 2. The 'field' data is that which was obtained for Table 1 from separate leaflets of the same collection.

The results of these experiments confirm the conclusion reached after a study of the data in Table 1.

Mr W. C. Moore has kindly drawn my attention (*in litt.*) to the fact that the pycnospores of his first collection of *Septoria lactucae* Pass. gave an average length of 27·5μ 'but after a week of dull or wet weather most of them were appreciably longer' (Moore, 1940), with an average length of 35μ. Is it possible that such slimy pycnospores behave just the reverse to the dry blastospores of *Ramularia onobrychidis* All. and *R. vallisumbrosae* Cav., and increase in length during wet weather as do the conidia of *Cercospora* species? Another interesting observation was made by Weber (1922) on the pycnospores of *Septoria tritici*. He found that the pycnospores collected in winter were considerably larger than those collected in summer. The summer spores measured '1·75 to 2·7μ by 39 to 70μ, averaging 2·2 by 50μ' and the winter spores measured 2·5–3·5μ by 52–85μ with a mean of 3 by 76μ.

Table 1. *Summary of collections of* Ramularia onobrychidis, *1943–5*

Date	Location*	Septation†	Humidity	Range in length (μ)	Mean length (μ)	Herb. I.M.I. no.
2. xii. 43	Ll.-M.	0 (1.2) 3	Dry	14·4–36·0	25·2	15,252
18. iii. 44	Gileston	0 (1.2) 3	Dry	15·3–36·0	24·5	—
18. iii. 44	S.down	0 (1) 2.3	Dry	10·8–36·9	25·8	—
11. viii. 44	Cardiff	0.1 (2) 3.4	Very dry	14·4–41·4	29·5	15,253
29. ix. 44	S.down	0 (1) 2.3	Moist	12·6–40·5	22·6	15,254
2. x. 44	Ll.-M.	0 (1) 2.3	,,	12·6–34·2	22·4	4482
31. x. 44	S.down	(0.1) 2	,,	10·8–38·7	22·0	15,255
31. x. 44	Ll.-M.	(0.1) 2	,,	13·5–32·4	21·4	15,256
23. xi. 44	S.down	(0.1) 2	Very moist	12·6–32·4	20·4	15,257
23. xi. 44	Ll.-M.	(0) 1.2	,,	11·7–29·7	21·0	—
12. xii. 44	S.down	(0.1)	Moist	12·6–25·2	18·9	15,258
22. xii. 44	S.down	(0.1) 2	,,	10·8–33·3	21·9	15,259
22. xii. 44	Ll.-M.	(0.1)	,,	10·8–27·9	19·4	15,260
5. i. 45	S.down	(0.1) 2	,,	11·7–27·9	19·6	15,261
5. i. 45	Ll.-M.	(0.1) 2	,,	9·9–26·1	20·1	15,262
9. ii. 45	S.down	(0.1) 2	,,	10·8–33·3	20·7	15,263
9. ii. 45	Ll.-M.	(0) 1	,,	14·4–25·2	19·8	15,264
22. ii. 45	S.down	(0.1) 2	Dry	9·9–36·0	22·1	15,265
22. ii. 45	Ll.-M.	0 (1) 2.3	,,	12·6–39·6	19·9	15,266
7. iii. 45	S.down	0 (1) 2.3	,,	11·7–37·8	22·7	—
21. iii. 45	S.down	(0.1) 2	,,	10·8–36·0	20·9	15,267
25. iv. 45	Ll.-M.	(0.1) 2.3	Very dry‡	9·9–35·1	20·3	—
29. v. 45	S.down (*a*)	(0.1) 2.3	Moist	9·0–32·4	21·1	—
29. v. 45	S.down	0 (1) 2.3.4	Dry‡	11·7–39·6	22·5	—

* Llantwit-Major is shortened to 'Ll.-M.' and Southerndown to 'S.down'.

† The septation of the majority of the conidia in Tables 1 and 2 is given in brackets. Thus 0 (1.2) 3 indicates that the conidia in this collection ranged between 0- and 3-septate, but were mostly 1- and 2-septate.

‡ These collections were stored in tins for a day after collecting.

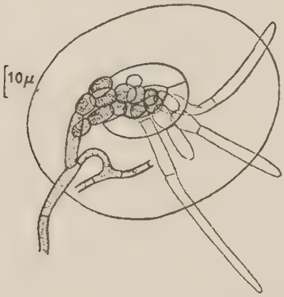
Table 2. *Effect of humidity on spore septation, range in length and mean length* (μ)

Source	Date		Septation	Range	Mean
Gileston	18. iii. 44	(*a*) Field	0 (1.2) 3	15·3–36·0	24·5
		(*b*) Treated	(0.1) 2	11·7–29·7	20·4
Southerndown	18. iii. 44	(*a*) Field	0 (1) 2.3	10·8–36·9	25·8
		(*b*) Treated	(0.1)	7·0–28·8	17·3
Southerndown	23. xi. 44	(*a*) Field	(0.1) 2	12·6–32·4	20·4
		(*b*) Treated	(0.1)	11·7–27·9	19·1
Southerndown	22. xii. 44	(*a*) Field	(0.1) 2	10·8–33·3	21·9
		(*b*) Treated	(0) 1	10·8–28·8	17·4
Southerndown	5. i. 45	(*a*) Field	(0.1) 2	11·7–27·9	19·6
		(*b*) Treated	(0) 1	10·8–27·9	16·6
Llantwit-Major	5. i. 45	(*a*) Field	(0.1) 2	9·9–26·1	20·1
		(*b*) Treated	(0) 1	11·7–21·6	15·4

If such significant variation in spore measurement wrought by climatic conditions is common, then diagnoses must of necessity be based upon numerous collections made throughout the year.

SCLEROTIA

Mature sclerotia consist essentially of a subglobose 'body' with a short 'neck' protruding through a much dilated stomatal aperture and slightly raised above the general level of the epidermis. The 'neck' is often markedly constricted where it passes between the guard cells. Sclerotia vary from 38 to 101 μ in diameter, and are generally produced towards the margin of a lesion (Pl. VI, fig. 3). They are formed under both upper and lower epidermis.



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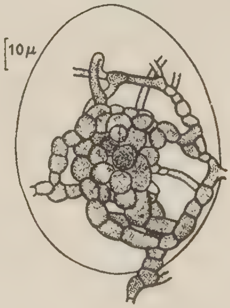


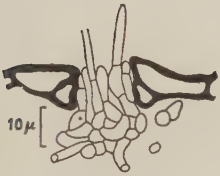
Text-fig. 12.

Text-fig. 11. Portion of strip of lower epidermis showing sclerotial initial in the substomatal cavity. The primordial cells next to the guard cells have already proliferated to produce four conidiophores which protrude through the stomatal aperture. The hyphal cells are pale brown as indicated by the stippling, whilst the outer circle denotes the lateral and well-defined limits of the substomatal cavity. Collected from a fairly moist situation. Southerndown, 5 January 1945.

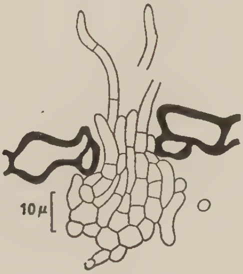
Text-fig. 12. Portion of an epidermal strip showing a young sclerotium seated in a substomatal cavity. All the cells are quite brown. Southerndown, 5 January 1945, from a fairly moist situation.

The 'body' consists of a homogeneous plectenchyma, the cell walls of which are somewhat thickened and brown, but the walls of the outermost layers of cells are only very slightly, if at all, more thickened and darker coloured than those of the core cells. The sclerotial 'neck' is characteristically composed of regularly radially arranged cells and resembles a very closely packed fascicle of pale brown and slightly thick-walled septate hyphae with rounded distal ends (Text-fig. 17). These 'neck' cells merge imperceptibly with those of the 'body'. Sclerotia were observed in small numbers in December 1943 and in abundance in January 1945, but this apparent difference is very probably due to more infection being seen in 1945 and more material being available for examination.

Development of sclerotia. The development of sclerotia has been followed from strips of epidermis of typical lesions and also from preparations of microtomed sections. A sclerotium arises from a single brown-walled hyphal branch which makes its way into a substomatal cavity. Here, it may or may not branch, but in either case proliferation occurs and a globose body is formed (Text-figs. 11–12). Further increase in size takes place by cell division and possibly by the addition of further hyphal branches on to it from surrounding mycelium. The body very soon becomes quite dark and opaque and in a preparation of an epidermal strip, examined under a low magnification, the young sclerotia are seen to be seated without exception within the almost regular confines of a substomatal chamber; this is far more readily observed in strips of the lower epidermis. While the 'body' is being produced, the cells nearest the stomatal aperture become proliferated and pierce the pore, which becomes dilated as more of the 'neck' tissue is produced (Text-fig. 14). Even at this stage of development the mesophyll and palisade cells are not completely disintegrated and in particular the outlines of the mesophyll cells, with their brownish disorganized contents, are quite conspicuous. The hyphae are entirely brown-walled at this stage and staining is not necessary to follow the development of sclerotia in epidermal strips.



Text-fig. 13.



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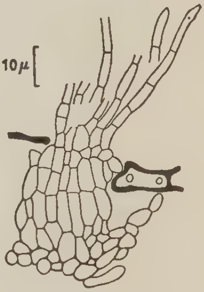


Text-fig. 15.

Text-fig. 13. Transverse section of a sainfoin leaflet showing stage of development of a sclerotium. Two of the visible 'neck' cells have sprouted to produce conidiophores. Collected at Llantwit-Major, 2 December 1943.

Text-fig. 14. Another stage of development of sclerotium. Llantwit-Major, 2 December 1943.

Text-fig. 15. An incompletely developed sclerotium whose 'neck' cells show considerable sprouting. Llantwit-Major, 2 December 1943.

From August 1944 until March 1945 diseased material was collected at fortnightly intervals from Llantwit-Major and Southerndown to find out when the sclerotia begin to form under the local conditions. Development began at the end of November in material from both stations, but judged by complete lack of fully formed sclerotia, very little further advance by the initials took place until late December, when mature sclerotia were seen. On leaf spots collected on 5 January 1945 and especially 22 January 1945 and later, the typical rings of sclerotia were very common. Heavy frosts and snow were experienced in December 1944 and January 1945, a fact that may well have been contributory to the development of sclerotia in such large numbers.

Sclerotial cells possess contents which stain with haematoxylin, but a deeper colour is retained by cells of the 'neck'. Crushed sclerotia liberate numerous oil globules which run together very quickly as found by Gregory (1939) in *Ramularia vallisumbrosae*, but he found that the sclerotium 'body' cells did not stain with cytoplasmic stains, although the 'neck' cells were found to be full of protoplasm. In all the sclerotia I have sectioned, no sign of any internal differentiation has been seen. Material bearing sclerotia has been allowed to overwinter during two successive winters, one fairly mild (1943–4) and the other very severe (1944–5), but no difference in the internal structure of the sclerotia was found.

Germination of sclerotia. The sclerotia, whatever their true nature, germinate very readily in both field and laboratory to produce conidiophores and chains of conidia. Even sclerotial initials (Text-fig. 11) are frequently seen to sprout; in fact very few mature and fully formed sclerotia were obtained without the presence of some conidiophores coming away from the 'neck' cells. Sclerotia do not seem to require any resting period prior to germination. They do not possess a carbonaceous coat or husk as do those of *R. vallisumbrosae* and its absence may be one of the reasons for the ready germination.

The length of the conidiophores varies; in sclerotia which have sprouted naturally in the field it tends to be about the same as that of conidiophores developed in sporodochia, or often a little shorter. When sclerotia are kept under very moist conditions in the laboratory they become more elongated and may reach a length of 60μ. Sporodochial conidiophores bear up to seven scars, but those from sclerotia have never been seen to bear more than four: and it may be that conditions were not suitable for the development of more spore chains, or adequate nutrition may have been lacking. No differences could be observed in conidia from the two types of fructifications; those from sclerotia also range from phragmospores to amerospores, depending upon the degree of humidity. The 'neck' cells are pale brown and conidiophores hyaline; the clear line of demarcation indicating the end of the 'neck' cell wall and the beginning of the conidiophore wall can frequently be observed (Text-fig. 18).

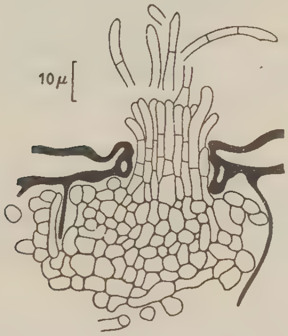
A sclerotial 'neck' similar to that found in *R. onobrychidis* has been illustrated and described by Killian (1928) for *R. sambucina* Sacc., thus: 'Ils [les sclérotes] se garnissent de prolongements filamenteux que j'interprète comme filaments conidifères, par analogie à ce que j'ai pu constater chez d'autres espèces.'

The sprouting of sclerotia to produce conidiophores and conidia is quite a common feature and has been recorded in *Sphaerella tussilaginis* Rehm (*Ramularia brunnea* Peck) by Wolf (1912), in *R. hieracii* (Bäum.) Jaap by Klebahn (1918), in *R. knautiae* (Massal.) Bubak by Laibach (1921), in *R. adoxae* Rabenh. and *R. geranii* (West.) Fuckel by Killian (1923), in *R. saxifragae* Syd. and *R. variabilis* Fuckel by Killian (1926), and in *R. vallisumbrosae* Cav. by Gregory (1939). Sprouting has also been observed in *Heterosporium gracile* Sacc. by Tisdale (1920) and in *Didymellina dianthi* Burt (*Heterosporium echinulatum* (Berk.) Cooke) by Burt (1936). There is, of course, no reason why the sclerotium should not sprout under moist conditions as it is purely vegetative tissue. In a similar manner perithecial walls have been seen to sprout in *Mycosphaerella tecomae* Wolf (*Cercospora sordida* Sacc.) by Wolf (1943).

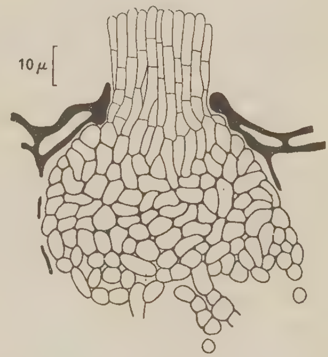
After sclerotia of *Ramularia onobrychidis* have produced a crop of conidia, and are crushed, no oil globules are liberated; presumably the oil has been used up in the production of conidia. Only one crop of spores could be obtained from sclerotia.

THE TYPE COLLECTION OF *RAMULARIA ONOBRYCHIDIS* PRILL. & DEL.

Through the kindness of Dr Arnaud and Prof. R. Heim, type material of the Prillieux and Delacroix name has been available for examination. It consists of an undated collection by G. Delacroix at Maine (Seine-et-Oise) and shows leaf spots similar to those described in this paper. No sclerotia were present and the collection is very probably a summer one. A portion of a leaf spot was teased apart and warmed in lactic acid with cotton blue. Thirty conidia gave a range of 8–33 × 2·5–5 μ with a mean of 19 × 4 μ; the conidia varied between 0- and 3-septate and were mostly 1-septate (Text-fig. 19). The conidia are narrower than those observed in my collections, but this discrepancy is due to the fact that the French material was mounted dead in lactic acid and the South Wales material was mounted in water in a fresh condition. Conidia from leaf spots of a collection from Llantwit-Major (2 October 1944) were mounted in lactic acid and cotton blue and thirty conidia were between 3·5 and 5 μ wide with a mean of 4 μ; the fresh collection mounted in water had conidia 3·6–6·3 μ wide with a mean of 4·7 μ. The conidiophores arise from substomatal sporodochia and reach a length of 40 μ and are 3–4 μ wide; they are geniculate or faceted. There is no doubt that the French material and my own are identical.



Text-fig. 16.



Text-fig. 17.

Text-fig. 16. An almost mature sclerotium showing parallel arrangement of 'neck' cells with rounded ends. Some sprouting has occurred. Llantwit-Major, 2 December 1943.

Text-fig. 17. A mature and unsprouted sclerotium. The 'body' is composed of a homogeneous plectenchyma with no indication of any internal differentiation. There is no definite outer layer of cells with thicker and darker walls to form a 'rind'. Llantwit-Major, 2 December 1943.

No type material of the name *R. onobrychidis* Allescher has been available, but I do not doubt that the two names apply to the same fungus.

A published exsiccatum of *R. onobrychidis* All. is cited by Lindau (1910, p. 768), 'Exs. Vill, *Fungi Bavar.* 882'; this is from the type locality, but it has not been available for examination.

ISOLATIONS

Cultures of the fungus were obtained from single phragmospores, didymospores, and amerospores and only slight differences were seen in the resulting colonies. In all, twenty cultures were started from material from different localities and collected at various times of the year, but in the following observations on the growth of the fungus on various media six isolations were used. Four of these were derived from summer infections and two from sprouting sclerotia. The media—Glucose agar, Glucose peptone agar, Czapek's agar, Oatmeal extract agar and Prune agar—were prepared as suggested by Gregory (1939). Observations were made on colonies on agar slants about six or seven weeks after inoculation and they were kept under laboratory conditions for this period.

Glucose agar. Colony of staling type, flat, maximum radius, 3–4 mm.; aerial mycelium very sparse, white to 'deep mouse gray'*; immersed mycelium 'dusky olive-green'; medium not noticeably pigmented; no sclerotia; no conidia.

Glucose peptone agar. Colony of staling type, raised, maximum radius 15 mm.; aerial mycelium very sparse, white to 'Chaetura drab'; immersed mycelium 'dull blackish green'; medium not noticeably pigmented; no sclerotia; conidia very few, unseptate.

Czapek's agar. Colony of staling type, flat, maximum radius 5 mm.; aerial mycelium scanty, 'Chaetura drab'; immersed mycelium 'dull blackish green'; medium not noticeably pigmented; no sclerotia; no conidia.

Potato dextrose agar (1 % dextrose, 2½ % agar). Colony of staling type, raised, maximum radius 22 mm.; aerial mycelium abundant, white to very pale 'flesh pink'; medium 'Dresden brown' around colonies; sclerotia few, black, immersed, scattered, sometimes confluent; conidia few, unseptate and 1-septate.

Prune agar. Colony of staling type, raised, maximum radius 30 mm.; aerial mycelium abundant, white to very pale 'flesh pink'; submerged mycelium of colonies dark brown centrally; medium not noticeably pigmented; no sclerotia; no conidia.

Oatmeal extract agar. Colony of staling type, flat, maximum radius 30 mm.; aerial mycelium scanty, white; submerged mycelium of colonies dark brown centrally; medium not noticeably pigmented; few imperfect sclerotia, dark brown to black; conidia very abundant, unseptate and 1-septate, mostly unseptate (7·0–25·0 × 3·5–5·5 μ, mean 15·6 × 4·6 μ).

Sainfoin agar (sainfoin leaf and stem decoction, 2½ % agar). Colony of staling type, raised, maximum radius 10 mm.; aerial mycelium abundant, white to very pale 'flesh pink'; medium too dark to observe pigmentation, but undersides of colonies dark brown centrally; no sclerotia; conidia abundant, unseptate and 1-septate.

On sainfoin agar, conidiophores were produced over the raised surface of the colonies and also on the sparse surrounding mycelium, more especially during drying out or where a colony was in contact with the glass surface.

Thirty conidia gave range of 8·5–22·5 × 3·5–5·5 μ with a mean of 14·1 × 4·1 μ. The mode of production of the conidia is shown in Text-fig. 20.

The conidiophores are short at first, but become elongate and septate; they arise at right angles from the sinuous surface hyphae and stand up erect on the medium although some conidiophores are very long and rest on the surface. Two or three conidial chains are produced, and branching of the individual chains frequently occurs. Complete chains of amerospores have been seen only in culture.

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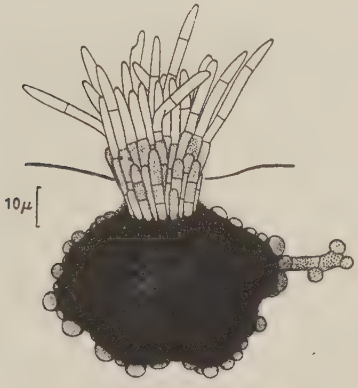
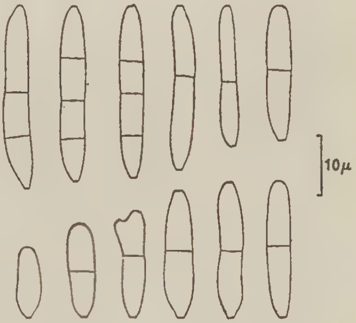
* All colours enclosed in single quotes were obtained by reference to Ridgway's *Colour Standards and Nomenclature*.



Text-fig. 18.



Text-fig. 19.

Text-fig. 18. Hyaline conidiophores produced from the pale brown (stippled) 'neck' cells of a sclerotium. Collected 2 December 1943 from Llantwit-Major, allowed to dry and sprouted in a moist atmosphere nine months later and cleared in potash.

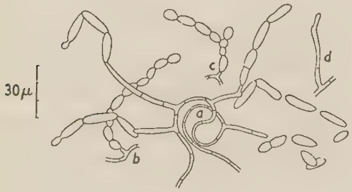
Text-fig. 19. *Ramularia onobrychidis*. Conidia from the type collection of '*R. Onobrychidis* Prillieux & Delacroix' (1893).

INOCULATION

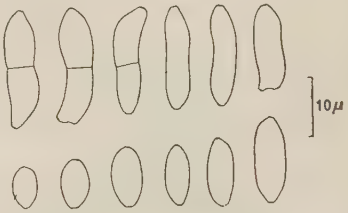
Difficulty was encountered in infecting sainfoin leaves successfully. When potted plants were spray inoculated with a spore suspension and kept under a bell-jar, the leaflets fell away in a few days. When a spore suspension was applied to the leaflets in the form of drops from a fine pipette the drops either rolled off immediately or fell off later with the slightest shock. Brushing the leaflets with a fine camel-hair brush tended to promote the adhering of the infection drop, but the leaflet might have been damaged by this treatment; in any case the leaflets were shed. In a few cases, however, the development of lesions on leaflets of detached leaves, inoculated with drops of spore suspension in a moist chamber, was observed and sporodochia were produced. Conidia from these sporodochia were planted on agar plates and typical colonies of *R. onobrychidis* resulted.

INTERPRETATION AND DISCUSSION

As this life-history study is based upon material collected from localities which are almost identical geographically, the interpretation of the life cycle may hold good only for this area. In Glamorgan, young leaf spots, possibly the result of recent infections, have been seen as late as November, and overwintering lesions bearing sclerotia can be seen producing conidia until February or March. It may be that conditions which permit the sprouting of sclerotia in the field also permit infection to take place. However, no new leaf spots were observed from early November 1944 until the end of March 1945, though spores were being produced from sclerotia in the intervening period. Possibly the spores remain viable on the surfaces of the leaves, as in *R. geranii-pusilli* (Killian, 1926), but this is not very likely because the young leaves which bear the spring infections were in bud during the preceding winter.



Text-fig. 20.



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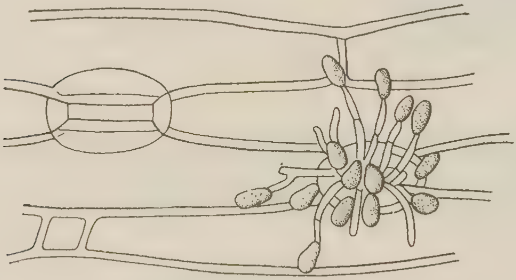

Text-fig. 22.

Text-fig. 20. Production of conidia in culture on sainfoin agar: *a*, sinuous surface hypha bearing four long conidiophores; *b–c*, two short conidiophores bearing chains of amerospores; *d*, a geniculate conidiophore.

Text-fig. 21. Conidia from seven-weeks-old culture on sainfoin agar.

Text-fig. 22. *Ramularia pusilla*. Reproduction, by camera lucida, of the original figure of *R. pusilla* by Unger, F. (1833, Tab. II, Fig. 12).

The dry spring of 1945 undoubtedly kept the leaf spot in check in the two four-year-old fields where a plentiful supply of diseased material was obtainable previously. At the end of May, however, a crop of English common sainfoin, sown in 1944 (Southerndown (*a*) in Table 1), came to my notice. It was a thick crop, ready for the first hay cut, and notwithstanding that the two previous months had been fairly dry the lower leaves were heavily spotted by the *Ramularia*; the dense leafy cover of the crop would, of course, conserve a moist atmosphere around the bases of the plants and promote infection.

Subject to further observations a measure of control of the disease would probably be obtained by taking an earlier hay cut to prevent spotting and shedding of all the lower leaflets; consequently the plants would dry out after cutting and further loss would be checked. It is rather interesting that the leaf spot should have been so widespread, and infection so heavy, in a crop of sainfoin in its second year; it is not impossible for the disease to have been seed-borne, or rather, pod-borne.

Dried leaflets bearing sclerotia have been stored for nine months indoors, but after this storage the sclerotia were still alive and sprouted in a moist atmosphere (Text-fig. 18). Provided that sclerotia remain dry they will not sprout, but in any case in winter the low temperature probably causes conidial production to be sluggish and protracted even under suitable moist conditions.

From the work of Killian (1923, 1926, 1928) and Gregory (1939) alone, it is clear that sclerotia may be (*a*) perithecial initials which have not experienced and received the essential meteorological and nutritional conditions necessary for the ultimate development of asci; (*b*) perithecial initials which have lost their ability to produce asci; (*c*) initials of pycnidia producing slimy conidia as in *R. lampsanae*; or (*d*) simple vegetative aggregations of cells produced for overwintering and intended to serve in a vegetative capacity only or, in other words, overwintering sporodochia.

When their structure is considered it seems unlikely that the sclerotia of *R. onobrychidis* represent any of the first three categories. In Glamorgan it has already been shown that for two years sclerotia have given no indication that they are other than cellular aggregations on which a crop of conidiophores and conidia is borne. It is possible that in other countries or under suitable climatic conditions in this country some of the sclerotia may develop into perithecia, but no one has yet described a perfect stage.

A comparison of sporodochia and sclerotia is very interesting as it shows that both arise substomatally beneath both upper and lower epidermis. Furthermore, sclerotia, like sporodochia, are quite undifferentiated internally and are very disposed to sprout even from their first formation with the conidiophores piercing the stomatal pore as with sporodochia. Sclerotia differ from sporodochia only in their larger size, their brown-walled cells and the presence of oil globules; sporodochia do not seem to store oil in this way. These facts suggest that the sclerotia of *R. onobrychidis* are merely winter forms of sporodochia and are the means of overwintering. It is pertinent to note here that the first sporodochia found in the spring of 1945 were distinctly pale brown, but otherwise similar to the hyaline sporodochia met with later in the season.

Slimy conidial stage. Following Klebahn, Mason, and Gregory, an attempt has been made to determine whether there exists in *R. onobrychidis* a slimy conidial stage in the form of a pycnidium. Immature minute (?)pycnidia up to 100 μ in diameter, which lacked spores, have been found in sections of leaflets infected with *Ramularia*, but these were not directly mixed with the sclerotia; it is only in leaflets bearing these overwintering bodies that they have been seen. They begin as a knot of brown-walled hyphae generally in the substomatal cavities, sometimes embedded in the disorganizing leaf tissues, but there is no indication of any terminal proliferation as with sclerotia. The core cells stain deeply with haematoxylin, indicating dense cytoplasmic contents; a cavity arises in the centre, but whether this was lysigenetic or schizogenetic could not be determined. In the most mature (?)pycnidium seen (Pl. VI, fig. 6) the original thick brown wall had been reduced to one or two layers of brown-walled cells lined by a thicker layer of deeply staining cells. From this inner lining, branching hyphal strands stretch through the cavity in all directions. A slight papilla is formed and an ostiole develops at the apex. This structure may belong to *Ramularia* or another fungus might have been present.

A *Phyllosticta* has been observed twice on sainfoin leaflets, with conidia measuring $4{\cdot}7–7{\cdot}5\,\mu \times 2{\cdot}0–3{\cdot}0\,\mu$ with a mean of $5{\cdot}5 \times 2{\cdot}5\,\mu$. In culture the *Phyllosticta*, which has not been assigned to any species, in no way resembled cultures of *Ramularia* and there is no reason to assume any genetical connexion between the two. Inoculations carried out with the *Phyllosticta* were unsuccessful, and it is presumably a saprophyte.

Prillieux and Delacroix (1893) thought that their *Ascochyta orobi* var. *onobrychidis* which attacks sainfoin was genetically related to their *Ramularia*. Numerous cultures of an *Ascochyta* from sainfoin, presumably identical with that under consideration by these authors, have been made; the two fungi have often been found together on the same leaflet, but they are distinct. *Septoria orobina* Sacc., another imperfect fungus found on sainfoin, also appears to be distinct from *Ramularia onobrychidis*.

Ascigerous stage. Some so-called species of *Ramularia* have been shown to be merely the conidial apparatus of different species of *Sphaerella* (Fr.) Rabenh. (*Mycosphaerella* Johanson). *Sphaerella onobrychidis* Hollós has been described from sainfoin (Saccardo & Trotter, 1913), but has not been recorded in Britain and it may prove to be based on the perithecium of one of the sainfoin parasites. Old and weathered leaves and stems of sainfoin have frequently been examined and the perithecia of five different species of Pyrenomycetes have been found since 1942. Only two of these have yet been identified: (*a*) *Pleospora herbarum* (Pers. ex Fr.) Rabenh., which is very common on old stems and leaves, and (*b*) *Phomatospora berkeleyi* Sacc. (syn. *Sphaeria phomatospora* Berk.), found on old sainfoin stems, Cardiff, April 1944; this has been cultured on various media, but its colonies show no resemblance to those produced by *Ramularia onobrychidis*. A species of *Leptosphaeria* that developed on dying leaves of sainfoin in the laboratory also differs from the *Ramularia* in culture.

THE ORIGINAL SPECIES OF *RAMULARIA* AND A HISTORY OF THE GENUS

Ramularia onobrychidis Allescher can be included comfortably in the genus *Ramularia* as delimited by Saccardo; however, this author was wont to supply his own circumscription to various genera with the exclusion of the type species. For this reason it was considered worth while to trace the history of the genus and to determine the fate of the original species.

In 1833 Unger described and figured two species of *Ramularia*, *R. pusilla* and *R. didyma*, with a brief generic description in German. A translation of the German runs as follows: 'In the same way as in "*Cylindrospora*" the higher formation of the entophytic hyphomycetes proceeds from the stomata of the epidermis. In this case, however, it is no longer the simple spores but added to these is a regular conidiophore which emerges in a more or less fasciculate manner from the stomates. We name this form *Ramularia* and so far, we have had the occasion to examine two species of the genus.'

The two species were described as follows: '*R. pusilla* (Tab. II, Fig. 12), floccis erectis, subramosis, sporidiis pellucidis ovalibus, minutis, auf missfarbenen Flecken der *Poa nemoralis*' and '*R. didyma*, floccis erectis, articulato infractis, subramosis, sporidiis ovato-cylindricis, didymis, auf Blättern von *Ranunculus polyanthemos* Lk. (Tab. II, Fig. 10, *a*).'

According to Wollenweber (August, 1913), Solheim (1929) and the Nomenclature Committee of the British Mycological Society (*Trans. Brit. mycol. Soc.* XXIV, p. 292, 1940) Unger did not describe the genus *Ramularia*; but this does not seem to be the case. There is a generic description and accordingly *Ramularia* may rank for priority from 1833 and not from Corda in January 1842 (*Icones fungorum* v, Prague).

Text-fig. 22 is a camera lucida reproduction of Unger's figure of *R. pusilla*. Nomenclators of the two original species are given below in chronological order.

Ramularia pusilla Unger, in *Die Exanthema der Pflanzen*, Vienna, p. 169, 1833.
≡ *Caeoma pusilla* (Unger) Bonorden, in *Handbuch der allgem. Myk. als Anleit....* Stuttgart, p. 323, 1851.
≡ *Ovularia pusilla* (Unger) Saccardo, in *Fungi ital.* t. 970, 1881.
≡ *O. pusilla* (Unger) P. A. Saccardo & D. Saccardo, in *Sylloge fungorum*, XVIII, p. 531, 1906.
≡ *O. pusilla* (Unger) [Lindau], in Rabenhorst's *Kryptogamen-Flora*, Band I, Abt. VIII, p. 235, 1907.
Type collection: not specified.
Locus natalis: on discoloured spots of *Poa nemoralis* (Gramineae).
Locus classicus: not stated, presumably Germany.

Ramularia didyma Unger (loc. cit.).
≡ *Didymaria Ungeri* Corda, in *Anleit. z. Stud. der Mykologie.* Prague, p. 199, August 1842.
≡ *Puccinia didyma* [(Unger)] Bonorden (op. cit.), Taf. I, fig. 23, 1851.
≡ *P. decumbens* Bonorden (op. cit.), p. 324, 1851.
≡ *P. Ungeri* [(Corda)] Bonorden, in *Zur Kenntnis einiger der wichtigsten Gattungen der Coniomyceten und Cryptomyceten.* Halle, p. 53, 1860.
≡ *Didymaria didyma* (Unger) Pound, in *American Naturalist*, XXIII, p. 163, 1889.
≡ *D. didyma* (Unger) Schroeter, in *Kryptogamen-Flora von Schles.* III, 2, p. 484, 1897.
Type collection: not specified.
Locus natalis: leaves of *Ranunculus polyanthemos*.
Locus classicus: not stated, presumably Germany.

In January 1842 Corda (op. cit. p. 7) redescribed the genus as follows: '*Ramularia* Unger Exanth. Taf. II, fig. 12 emend. Flocci entophyllini, repentes, continui, dein erumpentes. Sporae acrogenae, simplices; nucleo firmo.' Corda included only Unger's first species in the emended genus. In view of these facts *R. pusilla* should be considered the type species.

Fries (1849, *Summa Veg. Scan.* p. 493) described '*Ramularia* Ung. Fr.' as follows: 'Flocci discreti, e mycelio repente (subentophyllo) surgentes, ramosi, vulgo septati. Sporae solitariae, terminales, septatae.' Of the two original species *R. didyma* was included but not *R. pusilla*.

In 1851 (p. 41) Bonorden stated that '*Ramularia pusilla* Unger gehört ebenfalls zu dieser Gattung [*Caeoma*] wie ein Vergleich der Fig. 19 mit Fig. 42 [*Caeoma cinnamomeum*] deutlich zeigt'. Bonorden's fig. 19 is clearly a reproduction of Unger's original of *R. pusilla* and in the 'Erklärung der Abbildungen' fig. 19 is described as '*Caeoma* (*Ramularia* U.) *pusillum*....' This is a validly published new combination which may be cited as *Caeoma pusillum* (Unger) Bonorden. In 1860 (p. 11) Bonorden again takes up *C. pusillum* and cites *Ramularia pusilla* Unger as a synonym. He saw no grounds for allowing the genus *Ramularia* to stand as one (of the original) species had simple spores and the other two-celled spores, the latter having been taken up by Corda in 1842 as the original species of another genus. Furthermore he stated that *R. pusilla* stood in complete agreement with *Caeoma* in all essential characteristics.

In 1865, in *Fungi europaei* No. 874, Rabenhorst distributed a fungus on leaves of *Alchemilla vulgaris* under the name of *Ramularia pusilla* Unger. This is the first published exsiccatum called *R. pusilla*.

After 1833 numerous species of *Ramularia* were described with conidia indicated as being septate or non-septate. In 1880 (*Michelia*, II, p. 20) Saccardo redescribed *Ramularia* Unger and cited *R. urticae* Ces. and *R. cynarae* Sacc. as examples. Later, in 1886 (*Syll.* IV, p. 196), he claimed that he had emended the genus. Also in 1880 (op. cit. p. 17) Saccardo started off the genus *Ovularia* and then in 1881 (*Fungi ital.* t. 970), he made the new combination *O. pusilla* (Ung.) Sacc., and like Rabenhorst before him in 1865, he described the fungus as occurring on leaves of *Alchemilla vulgaris* and not *Poa nemoralis* the type host as given by Unger.

Spegazzini (1879 in *Decad. mycol. Ital.* No. 105) had already described *Ramularia aplospora* on *Alchemilla vulgaris* and this was cited as a synonym of *Ovularia pusilla* by Saccardo in 1882 (*Michelia*, II, p. 547).

Magnus (1904) drew attention to the confusion and made the new combination *O. aplospora* (Speg.) Magnus for the fungus on *Alchemilla vulgaris*. Then, in 1906, P. A. Saccardo and D. Saccardo (*Syll.* XVIII, p. 532) pointed out that the fungus bearing the name '*Ovularia haplospora* (Speg.) P. Magn.' was the same as that on *Alchemilla* described by Saccardo (1886, *Syll.* IV, p. 140) as *Ovularia pusilla* and also identical with that issued by Rabenhorst (1865) as *Ramularia pusilla* in *Fungi europaei* No. 874 also on *Alchemilla*. P. A. Saccardo and D. Saccardo (*Syll.* XVIII, p. 531) reproposed *Ovularia pusilla* (Ung.) P. A. Sacc. & D. Sacc. for the fungus on *Poa nemoralis*, presumably intending this to replace *O. pusilla* (Ung.) Sacc. (1881, *Fungi ital.* t. 970). But this new proposal is quite unnecessary because *O. pusilla* (Ung.) Sacc. is a validly published alternative binomial for *Ramularia pusilla* Unger. The fact that, at its origin, it included an admitted misidentification does not matter—'an "incidental" transference has been made' (Ramsbottom (1934) has discussed a somewhat similar nomenclatural problem).

Lindau (1907, p. 235) was also aware that Saccardo had the wrong fungus and unnecessarily reproposed the combination *Ovularia pusilla* (Ung.) [Lindau].

An examination of specimens in the *O. pusilla* folder in Herb. R.B.G. Kew disclosed that four of the five collections are on *Alchemilla vulgaris*. Two of these were examined and are, presumably, representative of *Ramularia aplospora* Speg. The fifth collection is on *Festuca gigantea*, not *Poa nemoralis* the type host.

Unfortunately Unger gave no measurements for his *Ramularia pusilla*, but in any event the key to the identity of this species, and the typification of the genus, lies entirely with the collecting of such a fungus on the type host.

Fresenius (1863) and P. A. Saccardo & D. Saccardo (1906, *Syll.* XVIII, p. 531) have drawn attention to the fact that *Ramularia pulchella* Cesati described on *Dactylis glomerata* is reminiscent of, and closely related to, *R. pusilla*. Certainly *R. pusilla* should be re-collected and re-studied because, as the Rules stand at present, the type species must not be excluded from the genus however much the latter is expanded, restricted, or emended.

The second of the two original species, *Ramularia didyma* Unger, has also been the subject of a number of new combinations. In January 1842 (op. cit. p. 7) Corda excluded *R. didyma* from '*Ramularia* Unger emend. Corda'; he took up *R. didyma* as the type species of his genus *Didymaria* and he called it *D. ungeri* in August 1842 (op. cit. p. 199). In 1851 Bonorden copied Unger's figure of *Ramularia didyma*, but labelled it '*Puccinia didyma*' (Taf. I, fig. 23). In the 'Erklärung der Abbildungen' (p. 324), however, he proposed a nom.nov. for it as follows: '23 *Puccinia* (*Ramularia didyma*) *decumbens*, aus einer Spaltöffnung hervorbrechend.' In 1860 (op. cit. p. 53) Bonorden made the following citation: '*Puccinia Ungeri* syn. *Ramularia didyma* Unger. *Didymaria* Corda.' As far as I am aware neither *Puccinia decumbens* Bonorden, *P. didyma* (Unger) Bonorden nor *P. ungeri* [(Corda)] Bonorden has been used in subsequent literature.

Pound (1889), realizing that *Didymaria ungeri* Corda is an invalid name, made the new combination *D. didyma* (Unger) Pound. Unaware that this had already been done, Schroeter (1897, op. cit.) made the same combination thus: *D. didyma* (Ung.) Schroeter.

'*Cylindrospora*' is an orthographic variant of *Cylindrosporium* Grev. and was first employed by Unger (1833). It was used by Schroeter (1897, op. cit.) to replace *Ramularia* and he made new combinations to this genus.

In the *International Rules of Botanical Nomenclature*, Jena, 1935, p. 125, '*Ramularia* Fres. *Beitr.* (1863) 88, Sacc. *Mich.* II (1880) 20, non Ung. *Exanth.* (1833) 169.—T.: *R. lactea* [Desm. *Ann. Sc. Nat.* 3. sér. Bot. XIV (1850), 109, sub Fusisporio] Sacc. *Mich.* II. 549' is proposed for conservation and '*Cylindrospora* Schröt. *Pilz. Schles.* II (1897), 485, ex Grev.' is proposed for rejection.

Returning to the original references cited above we find that there is neither a '*Ramularia* Fres.' nor a '*Cylindrospora* Schröt.' to be found; these are miscitations.

This matter has been the subject of a contribution of the British Mycological Society Nomenclature Committee (*Trans. Brit. mycol. Soc.* XXIV, p. 292, 1940). The committee held that 'The reason for suggesting *Ramularia lactea* as the type is obscure'. Saccardo compiled this species, but made no mention of septation of the conidia, whilst Bommer & Rousseau (1884) made the combination '*Ovularia lactea* Sacc.' which is perhaps best cited as *O. lactea* [(Desm.)] Sacc. ex Bommer & Rousseau. In 1893 (p. 321) Massee made the combination '*Ovularia lactea* [(Desm.)] Mass.' Lindau (1907, p. 468) described the conidia of *Ramularia lactea* as usually non-septate.

The committee then stated: 'As Saccardo's conception of *Ramularia* has been generally accepted it would be convenient to attribute the "emended genus" to him.' The citation '*Ramularia* Sacc. *Michelia*, II, 20, (1880)' cannot be verified. Following Clements and Shear (1931) it was recommended that *R. urticae* Ces. (which was claimed to have pluriseptate conidia) be accepted as type species and not *R. lactea*; the reason given is that Cesati 'appears to have been the first to use *Ramularia* in the sense of Saccardo'. The committee accepted the exclusion of the two original species of *Ramularia* from the genus; on p. 293, however, they stated: 'In 1897 Schroeter (loc. cit.) adopted "*Cylindrospora* Grev." to replace *Ramularia* in the sense of Saccardo', and they held the view that 'As this excluded Greville's type species [*Cylindrosporium concentricum*], Schroeter's use is now invalid....'

Eleven exsiccati of *Ramularia urticae* Ces. are available and have been examined, as well as a fresh collection on *Urtica dioica* (April 1946 in Herb. I.M.I. no. 4900). In all specimens the preponderance of conidia are amerospores with only a few didymospores. No pluriseptate conidia were observed.

Saccardo's emended diagnosis of the genus *Ramularia* Unger runs as follows: 'Biophila. Hyphae breve vage ramulosae; conidia ovato cylindracea, varia, denique 2- pluriseptata (et interdum catenulata)—Ex. *R. Urticae* Ces. & *R. Cynarae* Sacc.' (*Mich.* II, p. 20, 1880.)

What then is included in the genus *Ramularia* Unger in Saccardo's *Sylloge fungorum*? Of over 440 species and varieties compiled, in 369 of the descriptions the septation of the conidia is clearly stated; in Table 3 these are separated into eight categories depending upon the septation.

Table 3

Conidia	continuous	64	species or	varieties
„	up to 1-septate	185	„	„
„	„ 2- „	42	„	„
„	„ 3- „	63	„	„
„	„ 4- „	9	„	„
„	„ 5- „	4	„	„
„	„ 6- „	1	„	„
„	„ 8- „	1	„	„

Saccardo did not, therefore, restrict the genus *Ramularia* to species with conidia 'denique 2- pluriseptata'; in fact, the species with continuous or with 0- to 1-septate conidia outnumber those with conidia 2- to pluriseptate. *R. lactea* and *R. urticae* find places with the majority of species.

In 1880 (*Mich.* II, p. 17) Saccardo described *Ovularia* as follows: 'Biophila; hyphae subsimplices, erectae, apicem versus conidia globosa v. ovoidea gerentes. Est *Ramulariae* analoga—Ex. *O. obovata* Fuckel, *O. sphaeroidea* Sacc., sub *Ramularia*.' In 1886 (*Syll.* IV, p. 140), however, he added that the conidia were 'rarius breve catenulata'. Besides species with globose or ovoid conidia, Saccardo compiled in *Ovularia* fungi whose conidia were described variously as lanceolate, oblong and even cylindrical or cylindrical-ellipsoid and rarely 1-septate; in addition, the conidia of some species are described as being catenulate. The genus *Ovularia*, therefore, is not homogenous and considering the descriptions applied to conidial contour there seems to be a gradation embracing species in both *Ramularia* and *Ovularia*. This is borne out by a consideration of the spore lengths in relation to breadths where these are given in the diagnoses.

Ellis and Everhart (1885) did not accept the genus and included all species of *Ovularia* in *Ramularia*. Pound and Clements (1896) were also inclined to consider it better to unite *Ovularia* and *Didymaria* with *Ramularia*.

Massee (1893) compiled the British species of *Ramularia* and *Ovularia*. He restricted *Ramularia* to those species with conidia '1- to many septate' and *Ovularia* to those with one-celled conidia, whilst some species in both genera were described with conidia in chains. British species appearing in Saccardo's *Sylloge* under *Ramularia* and described with non-septate conidia were transferred to *Ovularia* by Massee; *Ramularia lapsanae*, however, appears as '*Ramularia lapsanae*' (p. 345) and also as '*Ovularia lapsanae* Mass.' (p. 320).

Gregory (1939) pointed out that 'on its host plant *Ramularia Vallisumbrosae* certainly has the habit of one of the Tuberculariaceae'; the same is true of *R. onobrychidis* and of many other species of *Ramularia*. However, in Saccardo's classification *Ramularia* is placed amongst the Mucedineae. As in *R. vallisumbrosae*, the plechtenchymatous masses bearing conidiophores in *R. onobrychidis* 'must be regarded as fundamental in the economy of the fungus'. (Gregory discusses this matter in detail.) In *R. vallisumbrosae* and *R. onobrychidis* alike, dark hyphae and darker stromata occur; in Saccardo's classification, however, only the hyaline aerial conidiophores and conidia are considered.

The so-called species of *Ramularia* are generally held to be the conidial apparatus of species of the genus *Mycosphaerella*, but a *Ramularia*-like conidial apparatus is also known to be produced by some species of the genus *Entyloma* of the Ustilaginales. Marchal and Sternon (1924) described and figured how *E. oenotherae* produced tufts of short, hyaline and unbranched conidiophores. These emerged through the stomata and each conidiophore bore, apically and subapically, chains of septate and continuous conidia—evidently a dry conidial apparatus no different from what is generally assigned to *Ramularia*. The presence of chlamydospores which germinated to produce 'basidiospores' showed the fungus to be an *Entyloma*. Of eleven species of *Ramularia* which these authors investigated, chlamydospores were found in *R. armoraciae* Fuckel and *R. variabilis* Fuckel, but they were not observed to germinate. Höhnel (1924) erected the genus *Entylomella* for the conidia of species of *Entyloma* and *Doassansia*. He claimed that the genus *Entylomella* is synonymous *pro parte* with '*Cylindrosporium* Sacc. (non Greville)'; this is an invalid citation, as *Cylindrosporium* is not Saccardo's genus—*Cylindrosporium* was proposed by Greville, and Saccardo (1880, op. cit. p. 12) gave it his own circumscription and excluded the type species; however, Höhnel made seven new combinations to his *Entylomella*, all of which are really nomenclaturally superfluous.

Ciferri (1928) emended *Entylomella* and cited as synonyms '*Cylindrosporium* Sacc. p.p. (non Grev.)' and '*Ramularia* Fuckel, *Symb. Myc.* p. 361, 1869'. Fuckel, in this reference, listed and described species under *Ramularia* Unger not *Ramularia* Fuckel. Ciferri made three new combinations to *Entylomella* using (*a*) '*Ramularia oenotherae* Marchal & Sternon', (*b*) *R. armoraciae* and (*c*) *R. variabilis*. I find no trace of '*R. oenotherae* Marchal &

Sternon' in the literature, but in 1924 these authors did suggest that the conidial form they found produced by *Entyloma oenotherae* Marchal & Sternon might be identical with the fungus by the name of '*Ramularia oenotherae* Ivanoff'.

Wolf (1912) stated that the production of 0- to 3-septate conidia in *R. brunnea* in chains is 'characteristic of the genus *Septocylindrium* while in *Ramularia* the conidia are not in chains.'

Wollenweber (February 1913) cited '*Septocylindrium* Bonorden' as a synonym of *Ramularia* Unger. He stated that '*Ramularia* is a genus related to *Fusarium* but with conidia of cylindrical shape. Chlamydospores are present, but the ascigerous stage is unknown. It should not be confused with the ascomycete *Mycosphaerella*, which has similar conidia but no true chlamydospores. *Ramularia* contains wound parasites causing root troubles, stem anthracnoses, leaf spot diseases and fruit rots.' Wollenweber made the new combination '*Ramularia didymum*' (Harting) Woll. (syn. *Fusisporium didymum* Harting, *Fusarium didymum* (Harting) Lindau). The significant point of this new combination is that '*Fusisporium didymum* Harting' is a slimy spored fungus whereas in *Ramularia* the conidial chains are generally regarded as representing dry terminus thallospores which are produced in acropetal succession. In August 1913, Wollenweber published his own circumscription of the genus '*Ramularia* (Unger) Fries' so that it included fungi with chlamydospores, and conidia produced several in sequence and occasionally adhering in short chains at the end of a conidiophore. Such conidia are slimy and are produced in basipetal succession from the end of a phialide. In the same paper Wollenweber erected the new genus *Cylindrocarpon* to include fungi similar to those included by him in *Ramularia*, but which differed in the absence of chlamydospores. Later, Wollenweber transferred to *Cylindrocarpon* some of the chlamydospore-producing forms which originally he had included in *Ramularia*.

It is interesting to note that Bubák (1916) made the combination *Ramularia septata* (Bon.) Bubák (syn. *Cylindrium septatum* Bon., *Septocylindrium Bonordenii* Sacc., *S. septatum* (Bon.) Pound, *S. septatum* (Bon.) [Lindau]). Babák stated that this fungus differs from *Cercosporella* because of the catenate conidia, and from *Ramularia* because the conidia were up to 4-septate, He considered that it was impossible to erect a new genus [*Septocylindrium*] on these grounds.

Overholts (1940) held a view similar to that of Wolf when he said 'I have seen some evidence that this fungus [*Ramularia oxalidis* Farlow] would be better referred to *Septocylindrium*, since in a few cases the spores were seen in chains of two'.

Of the facts arising as a result of tracing the history of the genus *Ramularia* the most significant is that our knowledge of the type species amounts to very little; in fact but few of the 400 odd species of *Ramularia* in Saccardo's *Sylloge fungorum* have been studied in any detail to determine the seasonal variation. Until the full range of the variation displayed by *R. pusilla* is known, the generic name *Ramularia* cannot be consistently applied.

It appears that the so-called species of *Ramularia*, *Didymaria* and *Ovularia* produce a fascicle of hyaline conidiophores emerging through the leaf or stem epidermis or through a stomate, and in the few species known in detail these conidiophores arise from an internal stroma; single conidia or chains of conidia, hyaline, variously septate or non-septate, varying in shape from spherical to ovate, obovate or cylindrical, are borne on hyaline conidiophores. As far as is known at present it is doubtful whether these three generic names can be used exclusively.

SUMMARY

Ramularia onobrychidis causes a leaf spot of sainfoin in Glamorgan, Wales.

In field material, chains of conidia are produced on conidiophores of substomatal sporodochia from late spring to early autumn. Conidia vary from phragmospores through didymospores to amerospores; moist conditions promote the formation of shorter conidia with fewer septa, and very dry conditions some longer, somewhat narrower conidia with up to four septa.

Sclerotia develop in winter in the substomatal cavities and are arranged usually in a ring around an old spot. They germinate very readily in the field under moist conditions without any resting period and retain their viability for at least nine months indoors, when kept dry. Sclerotia consist essentially of a more or less globose 'body' of homogeneous plectenchymatic cells with brown and slightly thickened walls, and a 'neck' of regularly columnar cells. It is from the apices of these neck cells that conidiophores arise. Sclerotia store oil, which disappears during the production of a single crop of conidia.

Single spore isolations of the different spore forms from summer spots and from germinating sclerotia have given similar cultures. The disease was reproduced with difficulty when healthy sainfoin leaflets were inoculated with spore suspensions and *R. onobrychidis* was re-isolated from the spots.

No perithecial stage in the life history has been discovered and it is probable that the fungus overwinters, in South Wales, by means of its sclerotia. A (?)pycnidial form, found near spots caused by *Ramularia* may represent the slimy conidial apparatus.

The fate of the original species of *Ramularia* is discussed and a history of the genus is given.

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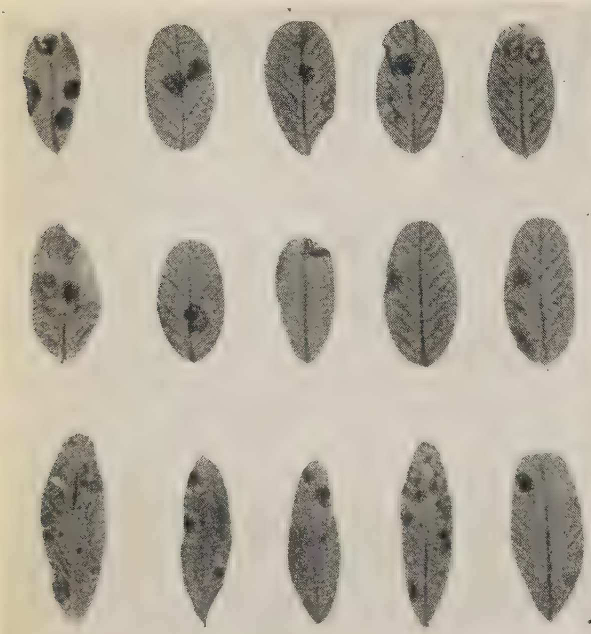

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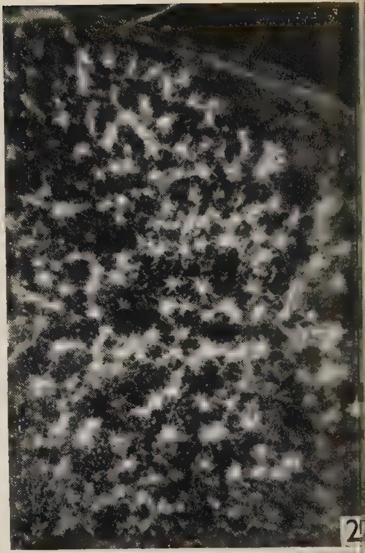

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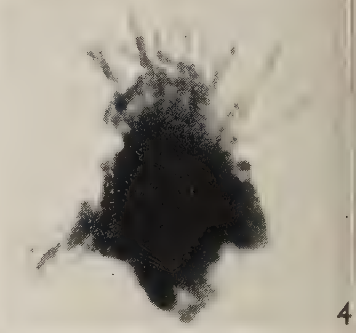

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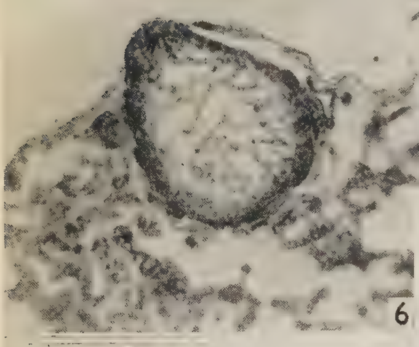

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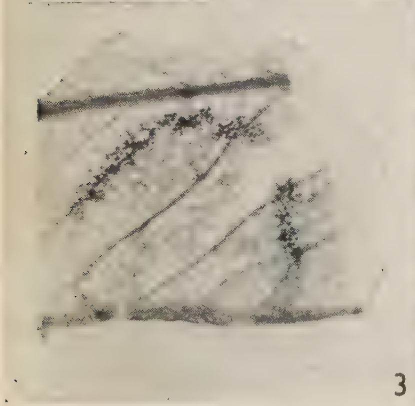

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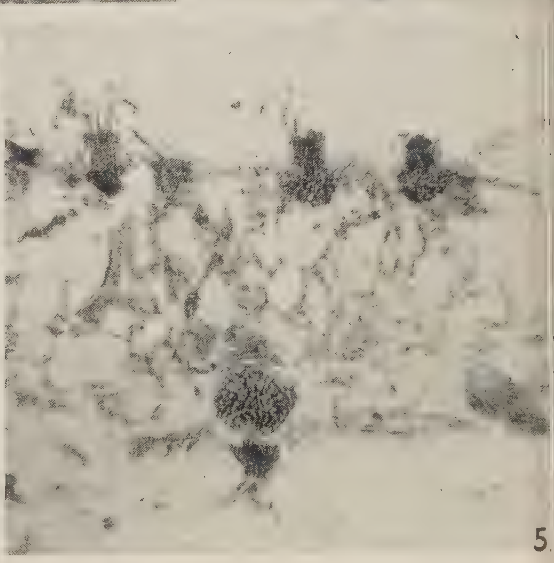

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EXPLANATION OF PLATE VI

Fig. 1. Sainfoin leaflets showing spots. Natural size.
Fig. 2. Surface view of sainfoin leaflets bearing conidiophores and chains of conidia.
Fig. 3. Portion of sainfoin leaflet, cleared in caustic potash, showing rings of sclerotia. Collected at Southerndown, January 1945. Unstained. × 7.
Fig. 4. Sprouting sclerotium teased from leaflet. Stained lightly with cotton blue. × 333.
Fig. 5. Portion of transverse section of leaf spot showing sprouting sclerotia. Collected at Cardiff, December 1943, and stained in haematoxylin. × 200.
Fig. 6. Young (?)pycnidium and (?)pycnidial initial from infected leaflet collected at Cardiff, December 1943. The branching strands can be seen stretching across the cavity. Stained in haematoxylin. × 333.

(*Accepted for publication* 19 *July* 1947)

POSTSCRIPT. Since this article went to press I have found that in 1836 Unger (*Ueber den Einfluss des Bodens*..., p. 224, Vienna) wrote as follows:

'144. *Ramularia pusilla* m. Char. gen.—Floccis erectis, subramosis stomatibus plantarum innatis, sporidiis simplicibus aut septatis.—Char. spec. Floccis erectis, subramosis, sporidiis pellucidis ovalibus minutis.—Auf missfarbnen Flecken der Blätter von *Poa nemoralis* im Spätsommer.—Conf. *Exanth. der Pflanzen*. Tab. II, fig. 12.

'145. *Ramularia didyma* m.—....'

In effect Unger here selected *Ramularia pusilla* as his lectotype for the generic name *Ramularia* Unger.

I notice, too, that Sprague (*Mycologia*, XL, 3, p. 309, 1948) has identified *Ovularia pulchella* (Ces.) Sacc. with '*Ovularia pusilla* (Ung.) Sacc. and D. Sacc.' and this species is recorded on *Bromus inermis* and *B. carinatus* in U.S.A.

STUDIES ON SOME DISEASES OF SAINFOIN (*ONOBRYCHIS SATIVA*)

III. LEAF-SPOT CAUSED BY *SEPTORIA OROBINA* SACC.

By S. J. HUGHES

(With 1 Text-figure)

A leaf spot of sainfoin caused by a species of *Septoria* was observed in June 1942 at the Cardiff University College Agricultural Experimental Plot at Ely. The disease was neither extensive nor, apparently, causing serious damage. Some pods of the few diseased plants bore mature pycnidia of the fungus. No further attacks by this fungus have been seen in any crops of sainfoin in Glamorgan. Fortunately, the fungus was obtained in culture at its first and only encounter and this permitted a study of its cultural characters and pathogenicity. The diseased spots were more or less round, up to 6 mm. in diameter, fawn and without a distinct margin. Pycnidia showed up as minute black dots on the spot; they were scattered but generally denser at the centre.

In culture on potato dextrose agar the *Septoria* produced a conspicuous cushion (or 'heaped-up' growth). The surface of the colony was much folded, black, with some white aerial mycelium. It was extremely slow growing and a colony seldom exceeded a diameter of 1·5 cm. even after some weeks at room temperature. The fungal cushion of closely interwoven, dark-walled hyphae was cheesy in consistency, and at the surface, pycnidia of irregular shape were produced in large numbers. After subculturing many times the fungus grew more readily on various media and covered, finally, a whole four-inch plate. The colony was then hyaline towards the margin, dark towards the centre and showed slight wrinkling of the surface. The potato dextrose agar in slants was coloured a very light 'pale Vinaceous-Lilac' (Ridgway), whilst the sediment in the medium sometimes became 'Vinaceous-Lilac'. Abundant pycnidia appeared as minute raised points on the surface and pycnospores developed freely and were exuded through the pores. Pycnospores were still viable after a year at room temperature in a dried culture.

No species of *Septoria* has previously been recorded on sainfoin and the following description, based upon the original diseased material, is provided.

Pycnidia amphigenous, mostly under the adaxial epidermis, denser at the centre of the spot, subepidermal, generally in the substomatal cavity, globose to pyriform, 48–128 μ in diameter, average 73 μ and mostly 68 μ, pale brown with darker-walled cells around an irregularly circular pore. Wall membranaceous composed of two to three layers of thick-walled cells. Pore 11–23 μ wide, average 15 μ, often borne on a short papilla, opening at the surface of the leaflet. Pycnospores hyaline, filiform, straight or sometimes slightly curved, ends rounded 15–40 μ long, average 27 μ, mostly 28 μ, about 0·8–1·0 μ wide, at first continuous and finely guttulate, later mostly 3-septate and distinctly so.

On living leaves and pods of *Onobrychis sativa* causing roundish, fawn spots with indistinct margins, up to 6 mm. in diameter. Cardiff, June 1942.

Measurements on 200 pycnospores made at intervals gave the following results:

Substrate and date	Range in length (μ)	Mean length (μ)	Length of most pycnospores (μ)
Sainfoin leaves, 1942	17–40	28	28
Sainfoin pods, 1942	15–35	26	28
Potato dextrose agar, 1943	18–33	26	27
Potato dextrose agar, 1943	22–37	30	30
Potato dextrose agar, 1944	19–40	28	28

The only *Septoria* recorded on species of the genus *Onobrychis* is *Septoria onobrychidis* N. Ranoïévich, on *Onobrychis saxatilis*. According to the description, as compiled by Trotter (1931), the pycnospores measured 28–51 × 2·5–3·5 μ; these measurements are far too large for the sainfoin fungus so the species need not, therefore, be considered further. The traditional course under such circumstances in the Coelomycetes is to describe the fungus as new. Of the species of *Septoria* recorded on herbaceous, papillionaceous hosts, one stands out, from its description, as being very similar to the sainfoin fungus. This species is *S. orobina* Saccardo (1878) and was described as follows: 'Maculis irregularibus arescendo subochraceis, atrocinctis; peritheciis remotis, punctiformibus, lenticularibus poro pertusis; spermatiis filiformibus, flexuosis, 30 × 0·75, eguttulatis, hyalinis.

Hab. in foliis Orobi verni in sylva Montello, Sept. 1874.'

No material of Saccardo's species has been available for examination, but his pycnospore measurements are so close to the mean I have obtained repeatedly for the *Septoria* on sainfoin that for the time being I prefer to call the sainfoin fungus *S. orobina* Sacc.

In culture the pycnidium of *S. orobina* Sacc. is symphyogenous in development; this can be observed in a culture of the fungus on dilute potato dextrose agar. Hyphae which are not morphologically different from others coil up irregularly to form a primordium. The mature pycnidium is spherical or pyriform with a pore situated on a papilla which may be short or long, depending, apparently, upon its depth of immersion in the medium. Two or three neighbouring pycnidia often develop in such close contact that their cavities merge into one, but separate pores are formed. The brown wall is complete all round, apart from the apical pore, and is composed of one to three dark-walled cell layers lined by thinner walled and hyaline sporogenous cells.

Pycnospores are also produced, in culture, on the individual hyphae of the mycelium (Fig. 1). They usually develop from a short lateral branch of a hypha. Groups of thirty or more spores were seen around a few short lateral branches, indicating that more than one pycnospore must have developed from each. The pycnospores are 3- to 7-septate, mostly 3-septate, but longer than those developed inside a pycnidium. The extra-pycnidial production of pycnospores in the genus *Septoria* has previously been observed and figured by Weber (1923), and it is not an uncommon occurrence when other Coelomycetes are grown in culture.

According to Weber (1923) some species of *Septoria* which he studied produced conidia from scant hyaline mycelium, whereas in other species no extra-pycnidial conidia were produced on the abundant mycelium.

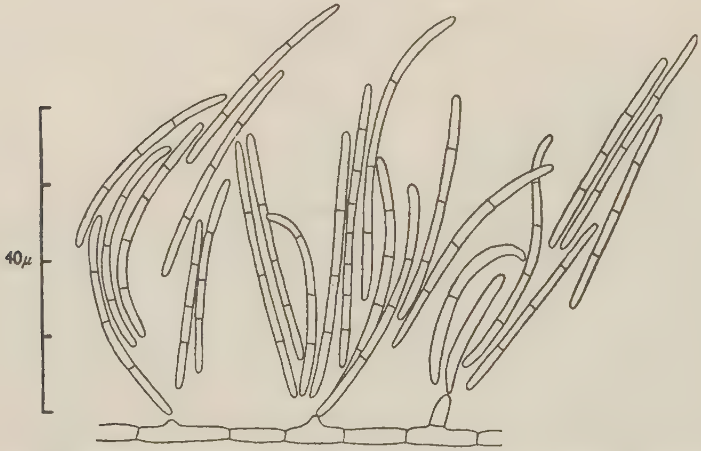

Fig. 1. *Septoria orobina* Sacc. Production of spores from a hypha in a culture on a diluted potato-dextrose-agar medium.

When sainfoin plants were spray-inoculated with a spore suspension, kept in a slightly moist atmosphere of a bell-jar for two days and then put outdoors, infection took place readily; most infected leaflets fell away after a few days. Pycnidia developed and spores were produced in ten to fourteen days. Microscopic examination showed that the germ tubes had entered through the stomata. Inoculations by spore suspensions were also made on damaged and undamaged leaves of white clover, red clover and *Vicia cracca* but no infection resulted. The fungus was still strongly pathogenic to sainfoin after two years in culture.

This investigation was carried out at the Advisory and Research Department in Agricultural Botany, University College, Cardiff, under the direction of Mr J. Rees. Preparations of the fungus have been deposited in the Herbarium of the Imperial Mycological Institute as No. 15,293.

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(*Accepted for publication* 22 *July* 1947)

THE PERITHECIA AND PYCNIDIA OF *LEPTOSPHAERIA NIGRANS*

By S. J. HUGHES

(With 2 Text-figures)

W. C. Moore (1943) stated that species of *Leptosphaeria* on cereals deserve further study and 'various names have been given to them, not all of which can be relied upon'.

In April 1944, at Colwinston, Glamorgan, *L. nigrans* was found in association with an *Ophiobolus* on cereal stubble. Perithecia of the *Leptosphaeria* were sunken between the veins of the darkened sheathing leaf base and bore numerous radiating brown hyphae 2–3·5 μ wide. Perithecia measured 180–330 μ in diameter when viewed from above, with a mean of 223 μ (twenty-three perithecia measured). The body of the depressed perithecium was dark brown and the short neck was black.

The more or less clavate asci were 74–109 μ long with a mean of 89 μ (eighty asci measured) and were 9–12·6 μ wide with a mean of 10·6 μ (thirty asci measured). The very pale brown and slightly curved ascospores were predominantly six-celled with the second cell from the apex always swollen and with the two end cells tapering to a rounded apex and longer than the intermediate cells. Ascospores measured 18–24 × 3·5–5 μ with an average of 21·5 × 4·1 μ (thirty-five ascospores measured). Permanent preparations of perithecia, pycnidia and pycnospores are deposited in Herb. I.M.I. as No. 15,288. Some material has also been deposited in the Herbarium of the Ministry of Agriculture, Plant Pathology Laboratory, Harpenden.

Desmazières' (1846) diagnosis runs as follows: '*Sphaeria* (caulicola) *nigrans*, Rob. in Herb. S. minuta, sparsa epidermide nigricante tecta. Peritheciis globosis vel sub-ellipticis depressis, albo farctis, basi villis brunneis vestitis. Ostiolo erumpente papillaeformi. Ascis clavatis; sporidiis fusiformibus curvulis, subhyalinis, 5-septatis.

Hab. in vagina Dactyli glomerati. Vere.'

Desmazières also made the following comments: 'Des taches brunes, quelquefois noirâtres, et qui se trouvent au printemps sur la gaine des feuilles sèches du *Dactylis glomerata*...seulement de très petits ostioles ponctiformes et d'un beau noir le percent et s'élèvent au-dessus. Ces périthéciums...n'ont pas plus de 1/5 de millimètre de grosseur....Les thèques ont environs de 1/10 millimètre de longeur, et s'épaississent insensiblement de la base au sommet...des sporidies,...d'une couleur olive extrêmement pâle, et longues de 1/50 de millimètre sur une épaisseur cinq fois moins considerable....Cette espèce curieuse et bien distincte a été récoltée au parc de Lébisey, pres de Caen, au mois d'avril 1843.'

Cesati and de Notaris (1863) made a new combination as follows: '*L*[*eptosphaeria*] *nigrans*- *Sphaeria nigrans* Roberg. in Desmaz. xiii. not. n. 25.

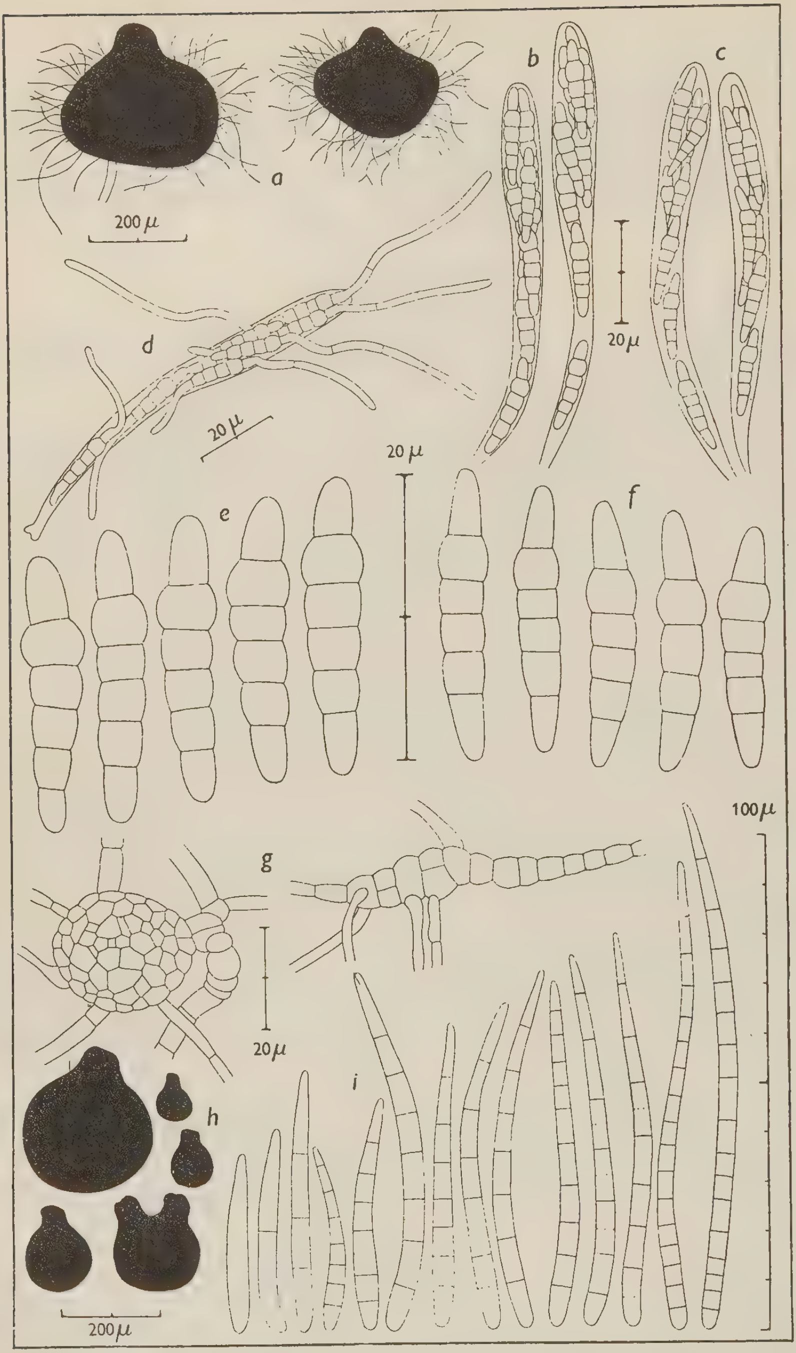

Fig. 1. *Leptosphaeria nigrans a, b, d, e, g, h,* and *i* were drawn from Herb. I.M.I. No. 15,288; *c* and *f* were drawn from French material cited in the text (Slide in Herb. I.M.I. No. 1838). *a*, perithecia; *b* and *c*, asci; *d*, ascospores germinating within the ascus; *e*, ascospores drawn in water; *f*, ascospores drawn in lactic acid; *g*, primordia of pycnidia drawn from culture on potato dextrose agar; *h*, pycnidia from culture on potato dextrose agar; *i*, pycnospores in various stages of development—from culture on potato dextrose agar.

e specim. a Cl. Lenormand. Berk. et Br. Brit. fung. n. 640. tab xi. fig. 27.' The legitimate citation is therefore *Leptosphaeria nigrans* [(Roberge in Desm.)] Ces. & de Not.

Through the kindness of the Director of the Royal Botanic Gardens, Kew, and of Miss Wakefield, I examined specimens which, if not actually a part of the type collection, are at least authentic for the name *L. nigrans*. This collection, 'Ex Herb. Berk. 1879', consists of five stems with sheathing leaf bases of *Dactylis glomerata* with the following label in a handwriting which has not been identified:

'*Sphaeria Nigrans* Roberg. (Desm 1774)

Sur la gaine des feuilles sèches du *Dactylis glomerata* Caen Roberge.'

Two perithecia measured 180 and 216 μ in diameter respectively. They appeared black in surface view and possessed brown hyphae, 2·5–4 μ wide, radiating from the perithecial wall. The epidermis is a little darkened and the papillate neck is only barely visible. Eight asci gave a range of 62–80 × 6·5–8 μ, whilst ten ascospores measured 16–21 × 3·5–4 μ and the second cell from the apex was constantly swollen. The ascospores are generally six-celled although one spore had only five cells.

Ascus and ascospore measurements of the Welsh material (Herb. I.M.I. No. 15,288) were made from water mounts of fresh material and those of the French material were made from lactic acid mounts, and this may account for slight differences in measurements. In glycerine jelly, however, the ascospores of I.M.I. No. 15,288 gave a range of only 17·5–21 × 3·5–4 μ.

Also in Herb. R.B.G. Kew is Desmazières *Crypt. France* No. 1774 issued as '*Sphaeria nigrans* Rob. in herb.' (Slide in Herb. I.M.I. No. 16,409.) This material is authentic for the name *Leptosphaeria nigrans* if not actually part of the original collection by Roberge. The fungus is described as occurring 'sur la gaine des feuilles du Dactylis glomerata'. Five perithecia removed from under the darkened epidermis ranged between 220 and 300 μ in diameter and bore brown hyphae radiating from them. Asci measured 64–90 × 6·5–8 μ and the ascospores 16·5–19 × 3–3·8 μ. The ascospores were mostly 5-septate with the second cell from the apex always swollen; occasional ascospores were 4-septate because the third cell from the apex had failed to divide.

The R.B.G. Kew specimen of Karsten's *Fungi Fennica Exsiccata* 962 was also examined (Slide in Herb. I.M.I. No. 15,173). This exsiccatum was issued as *L. nigrans*. The host is not identified but it is graminaceous. A single perithecium was 300 μ wide and 250 μ high. The ascospores measure 19–21 × 4–5 μ and are regularly 5-septate.

In all respects the four specimens mentioned above agree with each other sufficiently to be regarded as one species.

About fourteen ascus and ascospore isolations were made from I.M.I. No. 15,288. Ascospores germinated readily either inside or outside the ascus. All isolations produced pycnidia of the form-genus *Phaeoseptoria* Speg. (see Sprague, 1943) Pycnidia developed by the repeated septation of a hypha to form a spherical initial. Pycnidia in a three-week-old culture on potato dextrose agar varied between 56 and 176 μ with a mean diameter of 106 μ (150 measured); one pycnidium, however, reached a diameter of nearly 300 μ. The pycnidial walls were dark brown to black and fairly thick. Pycnospores arose from flask-shaped inner wall cells; at first hyaline they became pale brown, brown in mass, very variable in length with seven to sixteen distinct septa. Pycnospores with less septa than seven were probably immature. The following pycnospore measurements were obtained in culture:

Substrate	Age of cultures	Pycnospores measured	Range (in μ) Length Width	Mean (μ) Length Width
Potato dextrose agar	?	100	52·2–84·6 × 3–5	65·4 × 3·5
Potato dextrose agar	?	100	58·5–81·0 × 3–5	67·7 × 3·5
Potato dextrose agar	4 weeks	200	50·4–88·2 × 3–5	69·4 × 3·5
Potato dextrose agar	4 weeks	200	49·5–97·2 × 3–5	72·0 × 3·5
Steamed wheat leaves	?	200	61·2–108 × 3–5	82·8 × 3·5

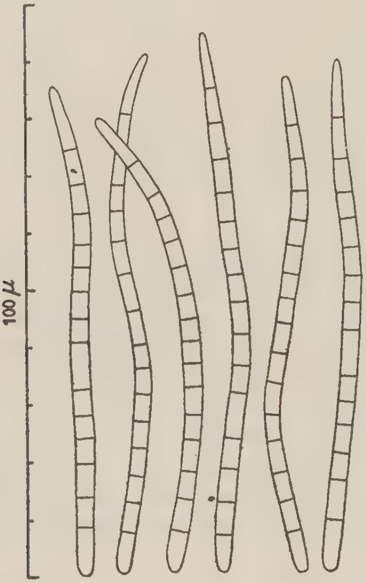

Fig. 2. *Septoria alopecuri* var. *calamagrostidis*. Mature pycnospores from the type collection in Herb. R.B.G. Kew. (Slide in Herb. I.M.I. No. 15,680.)

There is one species of *Septoria* in Grove's *British Stem- and Leaf-Fungi*, the description of which recalls the above pycnidial stage. It is *Septoria alopecuri* Syd. var. *calamagrostidis* Grove, which was described as follows: 'Spores linear, obtuse above, acute below, yellowish, 40–100 × 3–4 μ, faintly curved, with 3–13 distinct septa. On dead leaves of *Calamagrostis Epigejos*. Goosehill Woods, Worcs. (Rhodes).' (Fig. 2.)

The type collection in Herb. R.B.G. Kew has been examined (Slide in Herb. I.M.I. No. 15,680). A single pycnidium was mounted in lactic acid tinged with cotton blue and the pale brown pycnospores which were brown in mass measured 70–97 × 2·5–4 μ with a mean of 83 × 3·1 μ (thirty measurements). The pycnospores are generally curved but sometimes straight, and at maturity possess eleven to seventeen septa. Although the pycnospores of the type are very slightly narrower than those obtained in culture the similarity is very great; we have been warned, however, against comparing cultures with material on the host. Nevertheless, I am inclined to query *Septoria alopecuri* var. *calamagrostidis* as a synonym of the *Leptosphaeria nigrans*, and when Grove's variety is refound on the type host and cultured, further investigation will decide whether this assumption is correct or not. In view of the possibility it seems unnecessary to transfer Grove's variety to the genus *Phaeoseptoria* Speg. It is interesting to note that *Leptosphaeria nigrans* has been recorded on *Calamagrostis epigejos* in Denmark (Lind, 1913).

According to Bisby and Mason (1940) the name *Leptosphaeria nigrans* has appeared five times in British literature cited by these authors. It was first recorded for Britain (as *Sphaeria nigrans*) by Berkeley and Broome (1852), who described the fungus as occurring on *Dactylis glomerata* and figured six to eight septate ascospores with a more or less centrally placed swollen cell. Their description and the first British record is based on a collection 'ex Herb Berk' in Herb. R.B.G. Kew labelled thus: '21. *Sphaeria nigrans*, Desm. no. 1774. Batheaston. Feb. 3. 1851.' In this material the perithecia are visible as black pin-points at the surface of the epidermis and there is hardly any darkening of the host tissues. Three perithecia measured 140, 150 and 165 μ in diameter respectively and bore very few hyphae coming away from the black wall. Asci measured $65–80 \times 9–10\,\mu$ and ten ascospores measured $20–23 \times 3–4\,\mu$; the ascospores are constantly 5-septate with the fourth cell from above always swollen. The three upper cells are each shorter than each of the two lower cells so that the intermediate swollen cell appears centrally placed. This fungus is identified as *Leptosphaeria fuckelii* Niessl and a preparation is so disposed in Herb. I.M.I. as No. 15,327.

Niessl (1887) considered that Berkeley and Broome's description and figures of what they called *Sphaeria nigrans* were based on *Leptosphaeria fuckelii* but Niessl saw no material. Niessl points out the differences between *L. fuckelii*, and *L. nigrans*. He claimed to have examined type or authentic material of *L. nigrans*, viz. Desmazières, *Pl. Cr. de France*, No. 1424 (but not No. 1774), and found the ascospores to be only $16–17 \times 2{\cdot}5–3\,\mu$, but they were 5-septate with the second cell from the apex swollen.

Fries (1849) claimed *Sphaeria nigrans* as a synonym of his *S. culmicola*. It is impossible to confirm this until type or authentic specimens are available, because Fries described no ascospores.

Cooke (1871) gave an English translation of Desmazières' diagnosis of *S. nigrans* and cited his [*Crypt. France*, Ser. i] No. 1774; Cooke described the fungus as occurring 'on *Dactylis glomerata*. Feb.' I take this to be Berkeley's original record especially because Berkeley's comments are quoted as well, including the description of the ascospores with the 'middle joint' swollen.

Bucknall (1880) recorded '*Sphaeria nigrans* Desm.' from 'Portbury, June, 1879'. Unfortunately, Bucknall's herbarium was destroyed and unless he sent some of his material to some other mycologist and it is still in existence this record cannot be confirmed. There is no material from Portbury in Herb. R.B.G. Kew.

Cooke (1889) listed *S. nigrans* under *Heptameria* Rehm & Thüm. and the combination may be cited as *Heptameria nigrans* (Rob. in Desm.) Cooke. No specimens were cited.

Massee (1880) listed '*H*[*eptameria*] *nigrans*, Desm., Sacc. Syll. 3108; Hdbk. 2716 [Cooke, 1871]. On grass leaves. Shere, Neatishead.' In Herb. R.B.G. Kew there is a specimen ex Herb. M. C. Cooke labelled in Cooke's handwriting: '*Sphaeria nigrans*...immature...Neatishead'. The material consists of two pieces of a graminaceous host which is partly darkened. Four perithecia measured 190–220 μ in diameter, but no ascospores were found. This British record cannot be confirmed.

The 'Shere' specimen cited by Massee is undoubtedly that found in the *Leptosphaeria nigrans* folder in Herb. R.B.G. Kew (ex Herb. M. C. Cooke), and labelled thus in Cooke's handwriting: 'Dr Capron...Shere...402. Spores same size as *S. culmifraga* but resembling *S. nigrans*.' The *Leptosphaeria* which is present is distinct from *L. nigrans*, but the preparation (Herb. I.M.I. No. 16,053) has not been assigned to a species with satisfaction.

The cultural work was carried out in the laboratory of Mr J. Rees, Advisory Mycologist, University College, Cardiff.

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STUDIES ON BRITISH CHYTRIDS

VI. AQUATIC SYNCHYTRIACEAE

By HILDA M. CANTER
The Freshwater Biological Association, Wray Castle, Ambleside, and Department of Botany, Birkbeck College, University of London

(With Plates VII–XI and 13 Text-figures)

Aquatic representatives of the Synchytriaceae, apparently widely distributed, have not definitely been described from this country. The older British literature contains many records (Thwaites, 1846–7; Shadbolt, 1852; Smith, 1853) of spiny bodies (asterospheres), causing hypertrophy in members of the Conjugales, which may well have been prosori of *Micromyces* or *Micromycopsis* spp. The writer, while working at the Freshwater Biological Association's Laboratory, Wray Castle, found several species of these genera and a new genus *Endodesmidium*, which seems to be a primitive member of the family.

I. **Endodesmidium formosum** n.gen., n.sp.

In April 1946 what is believed to be a species of a new genus of the Synchytriaceae was found parasitizing a form of *Netrium oblongum* (De Bary) Lütkem with purple sap, in Rusland Moss (a Sphagnum bog), Lancashire.* In a second collection, made three weeks later, the same chytrid was found on *Cylindrocystis crassa* De Bary, and *C. brebissonii* Menegh. The organism appears to be new to science and a detailed account of its life history on *Netrium oblongum* follows.

The first sign of fungal attack is a disintegration of the host chloroplast, and the accumulation of fatty material along its central axis. The parasite is first visible as a large oil globule by the side of which smaller oil globules accumulate (Text-fig. 1*a* and Pl. VII, fig. 2). Owing to the dense algal contents the actual fungal protoplasm cannot be distinguished at this stage. With the accumulation of oil globules a naked oval thallus becomes visible (Text-fig. 1*b*, *c*, and Pl. VII, fig. 3). The contents of this gradually become more granular, but the large oil globule remains as a characteristic feature. Around this naked thallus a wall is secreted, forming a body equivalent to the prosorus of other aquatic Synchytriaceae, with the oil globule at the anterior end. The wall is usually coloured a brownish purple, possibly due to the precipitation of the pigment from the purple host sap; it rarely remains colourless and is always smooth. The number of parasites in each host cell varies from one to six, and their position is in no way connected with that of the nucleus. Whether, when more than one parasite is present in a cell, it is due to fragmentation of the fungal cytoplasm, or to separate

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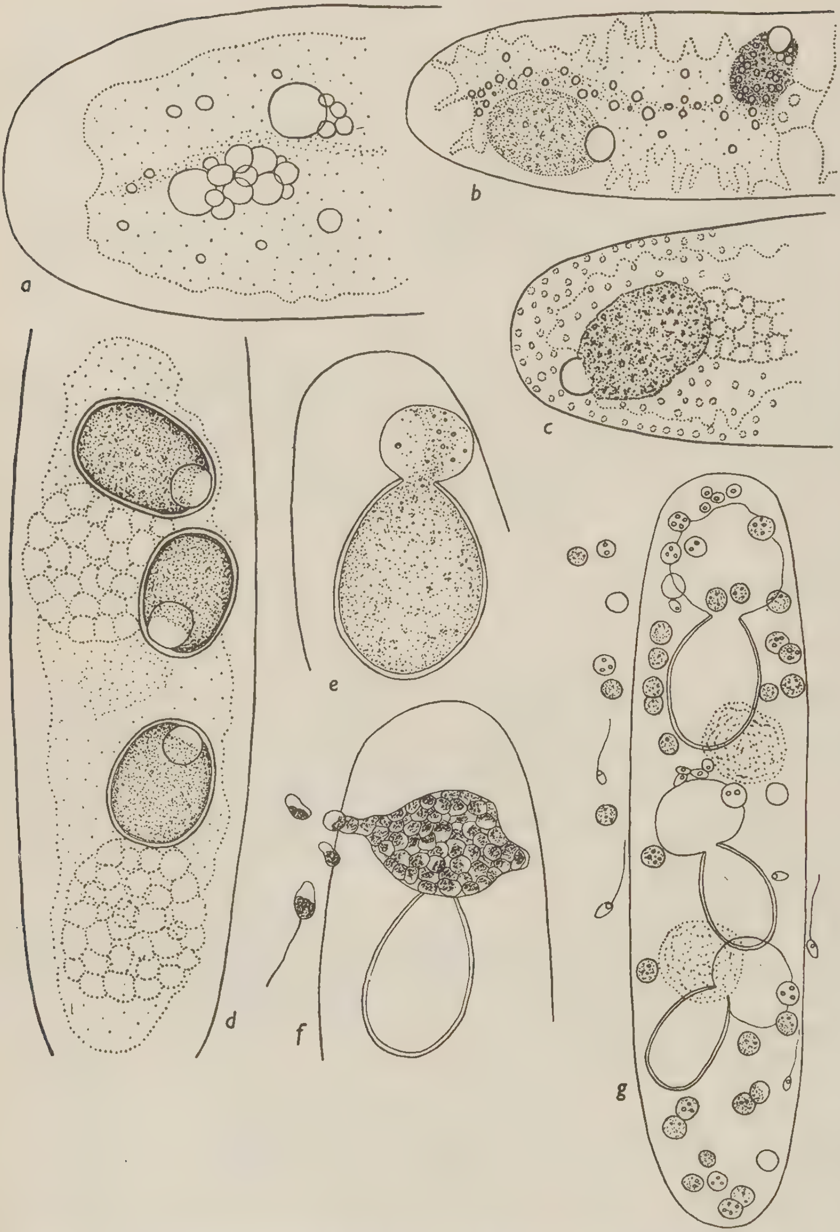
* This fungus was again collected in April 1947 from the same locality.



Text-fig. 1. *Endodesmidium formosum* n.sp. *a*, very young thalli with conspicuous oil globule and a few smaller globules posteriorly. ×1333. *b*, *c*, naked prosori each with a conspicuous anterior oil globule. *b*, ×700, *c*, ×1000. *d*, three mature prosori. ×975. *e*, germinating prosorus. ×975. *f*, mature sorus with non-swarming amoeboid swarmers emerging from it; the large oil mass of each swarmer is shaded and one specimen has a short posterior flagellum. ×975. *g*, three germinated prosori with dehisced sori; sporangia in various stages of development and actively motile zoospores both inside and outside the host cell. ×700.

infections is unknown. By the time the prosorus is mature the host chloroplasts are reduced to two yellowish oily masses, while the cytoplasm is greyish and much shrunken. The prosori are usually oval and vary from $16{\cdot}5 \times 28{\cdot}5$ to $10{\cdot}6 \times 15{\cdot}6\mu$.

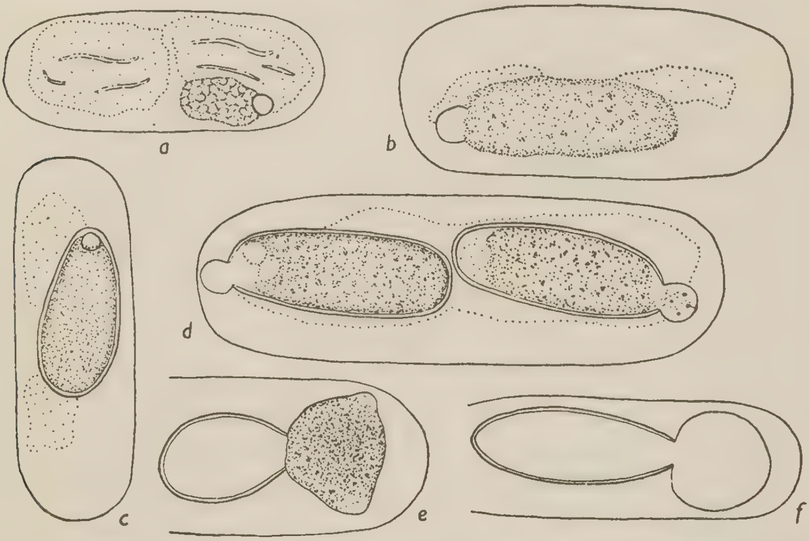
Germination of the prosorus readily takes place. A pore is formed at the anterior end, the oil globule loses its identity, and the contents squeeze out to form a thin colourless smooth-walled vesicle (the sorus) within the host cell (Text-fig. 1*e*, and Pl. VII, fig. 7). The sorus continues to swell as material gradually passes out from the prosorus leaving a clear area at its base. The mature sori are subspherical and range from 16–25μ high × 12–21μ broad. In older specimens which have dehisced and are completely empty, a projection of wall material at the apex of the prosorus is sometimes seen, suggesting that dehiscence of the prosorus is by a lid. The sorus when mature has two oppositely directed lateral papillae, which may or may not pierce the host wall. The soral content divides to form about fifty oily structures, 3–4μ in diameter, which ooze singly through the papillae, either into the external medium or into the cavity of the *Netrium* cell (Text-fig. 1*f*). These bodies are very sluggish, remaining quiescent or exhibiting amoeboid movement; free active motion was never observed. Three specimens only were seen with a single short posterior protoplasmic thread, possibly a flagellum. These bodies (sporangia) are soon all quiescent, the content becomes clearer and from two to five minute oil globules, indicating the position of the zoospores, can be distinguished (Text-fig. 1*g*, and Pl. VIII, fig. 1). The sporangia liberate from two to five normal chytridiaceous zoospores, 1μ in diameter, with a long flagellum. These small sporangia producing a few zoospores are very reminiscent of the cysts arising from the primary zoospores, which are liberated from resting spores in *Allomyces cytogenus* (Emerson, 1941).

The actual fate of the zoospores liberated from the sporangia of *Endodesmidium formosum* is unknown. They swim actively and are no doubt the dispersal phase of this organism. Almost certainly they reinfect the *Netrium*, although empty zoospore cases have never been observed on the outside of the host wall. If, however, the zoospores behave as recorded for *Synchytrium endobioticum* (Schilb.) Percival, by Curtis (1921), then no zoospore case would be visible, since the naked zoospore itself penetrates the host wall.

Several mature prosori were stained for the presence of a nucleus. In five specimens a densely staining area (Pl. VII, fig. 4*b*), probably the nucleus, was located at the base of the large anterior oil globule. This position is perhaps suggestive of a centre of organization, for in young stages, growth of the prosorus is accomplished by the addition of material behind the large oil globule. From this densely staining area a network of granules extended into the surrounding cytoplasm.

The occurrence of resting spores is very doubtful. After some material had been kept at 8–11° C. for about five weeks it was examined, and many ungerminated prosori were still present. They were structurally indistinguishable from those already described, except in having perhaps a slightly thicker wall. They germinated after they had been subjected to a temperature of 19–20° C. for a few days.

As mentioned earlier, this chytrid was also found on *Cylindrocystis crassa* and *C. brebissonii*. Although the actual emergence of the non-swarming zoospores (sporangia) was never observed, the sequence of events followed in its life history are the same as those described above. The prosorus of the chytrid in these desmids was rather longer and narrower, varying from 15 × 35 to 9 × 21 μ, and the subspherical sorus from 13 × 17 to 12·5 × 13 μ. This form is illustrated in Text-fig. 2*a–f* and Pl. VII, figs. 5, 6.



Text-fig. 2. *Endodesmidium formosum* n.sp. *a*, *b*, young naked prosori in *Cylindrocystis*; note conspicuous anterior oil globule. *c*, mature prosorus. *d*, two germinating prosori. *e*, empty prosorus with almost mature sorus; two dehiscence papillae just visible. *f*, dehisced sorus. *a*, *c*, × 700; rest, × 975.

Endodesmidium n.gen.

Thallus endobiotic, holocarpic, at first naked, later transformed into a smooth-walled prosorus; sorus endobiotic, thin walled, smooth, the content dividing into numerous bodies which emerge through papillae in the external medium or into the cavity of the host; sporangia spherical producing minute zoospores; zoospores posteriorly uniflagellate with a conspicuous oil globule.

Endodesmidium gen.nov.

Thallus endobioticus, holocarpicus, primo sine tunica, demum in prosorum laeve transformatus. Sorus endobioticus, tenuiter tunicatus, laevis, parte interiore in corpuscula numerosa per papillas emergentia partiente. Sporangia sphaerica. Zoosporae minutae, postice uniflagellatae, guttula oleosa distincta praeditae.

Endodesmidium formosum n.sp.

Prosorus oval, 28·5 × 16·5 to 15·6 × 10·6 μ, with a smooth, usually purple wall; sorus subspherical, 16–25 μ high × 21–12 μ broad, having at maturity two oppositely directed dehiscence papillae, the content dividing into about fifty bodies (4 μ in diameter) with a conspicuous mass of oil and rarely a single, short posterior flagellum (6–8 μ long), emerging through the papillae; sporangia spherical, 4 μ in diameter, discharging two to five minute spherical zoospores 1 μ in diameter, with a conspicuous oil globule and long posterior flagellum; movement active swimming; resting spore similar to prosorus, wall slightly thicker.

Parasitic in *Netrium oblongum*, *Cylindrocystis crassa*, and *C. brebissonii*, from Rusland Bog, Lancashire, England, in April 1946.

Endodesmidium formosum sp.nov.

Prosorus ovalis, 28·5 × 16·5 ad 15·6 × 10·6 μ, tunica laevi, plerumque purpurea. Sorus subsphaericus, 16–25 μ altus, 12–21 μ latus, maturitate papillis dehiscentiae duabus oppositis praeditus; pars interior in c. 50 corpuscula partiens; corpuscula 4 μ diam., massa oleosa distincta et rare flagello singulo 6–8 μ longo postice praedita, per papillas emergentia, pigre se moventes. Sporangia sphaerica 4 μ diam.; zoosporae minutae 1 μ diam., guttula oleosa distincta praeditae, postice longe uniflagellatae, libere natantes. Spora perdurans ut prosorus similis, tunica paullo crassiore. Hab. Parasiticus in *Netrio oblongo*, *Cylindrocystide crassa* et *C. brebissonii*, Rusland Bog, Lancashire, Angliae, April 1946.

There seems to be little doubt that this chytrid is a member of the Synchytriaceae and may possibly represent a primitive condition within the family, where the numerous sporangia emerge from the sorus as relatively large, possibly uniflagellate, amoeboid swarmers, which later encyst and produce a few uniflagellate chytridiaceous zoospores. Scherffel's (1926) investigations on *Micromycopsis cristata*, although not based on continuous observations, are extremely interesting in this connexion. He occasionally found that the sporangia were set free from the soral membrane before maturity of the zoospores. They emerged as relatively large (4 μ in diameter) uniflagellate (flagellum 6–8 μ long), non-swarming, amoeboid swarmers, with a single large oil globule 3 μ in diameter; they encysted and produced zoospores endogenously. These observations agree with those recorded for *Endodesmidium formosum*, where this is the normal procedure. Scherffel also states that on such rounded portions lying free in the water, which apparently correspond to the amoeboid swarmers, there appear sometimes, two fine thread-like unbranched flagella-processes, which he suggested might be rhizoids. The only structures resembling the latter found by the author were bacteria.

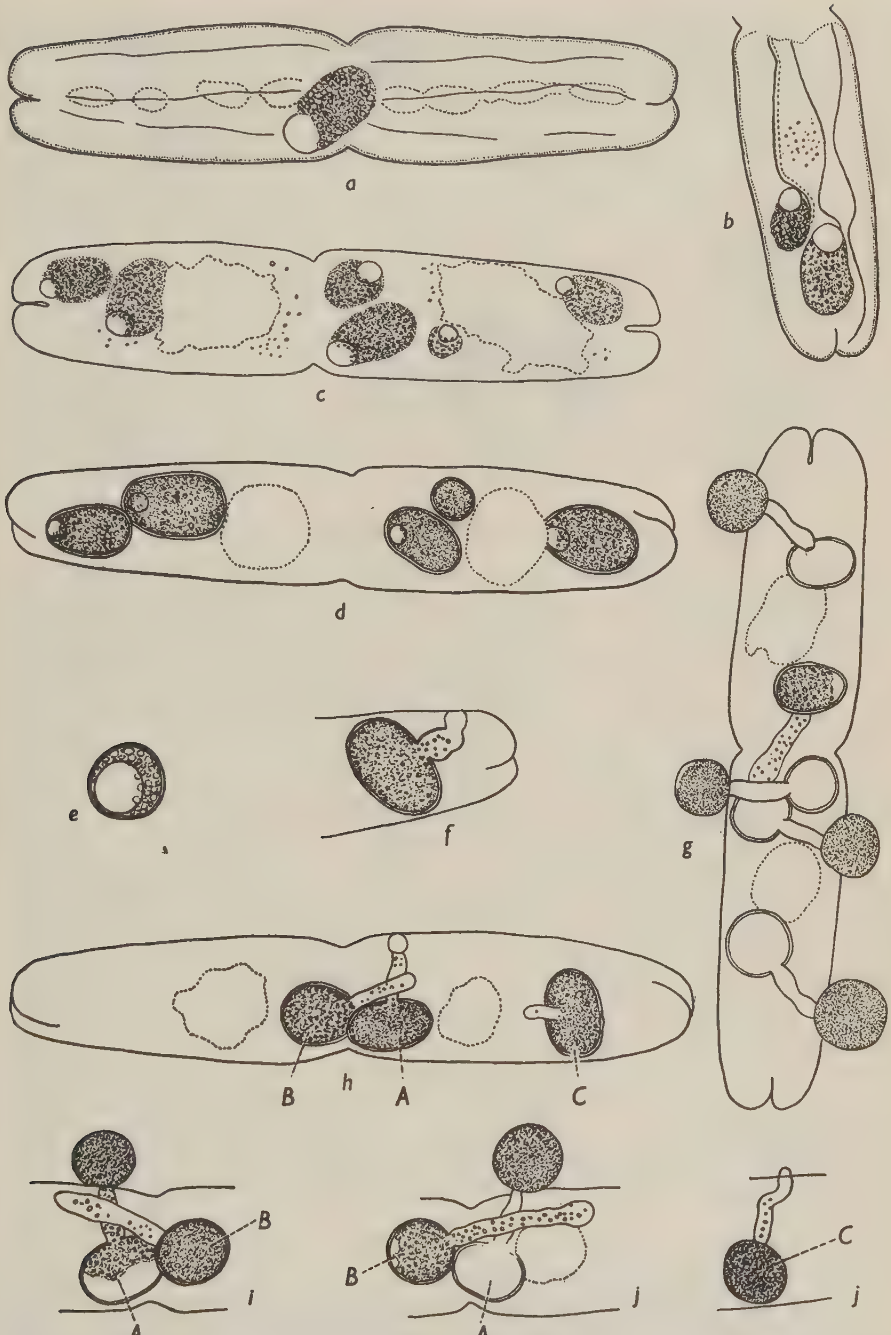
II. *MICROMYCOPSIS FISCHERI* SCHERFFEL

M. Fischeri was erected by Scherffel (1926) for an incompletely known fungus, differing from other species of the genus in its smooth, colourless soral wall. A similar organism was found by me during April and May 1947 parasitizing *Tetmemorus brebissonii* (Menegh.) Ralfs, from a path leading to Three Dubs Tarn, Claife Heights, near Sawrey, Lancashire. Although *T. granulatus* Bréb. was abundant, it, together with other desmids, was not attacked.

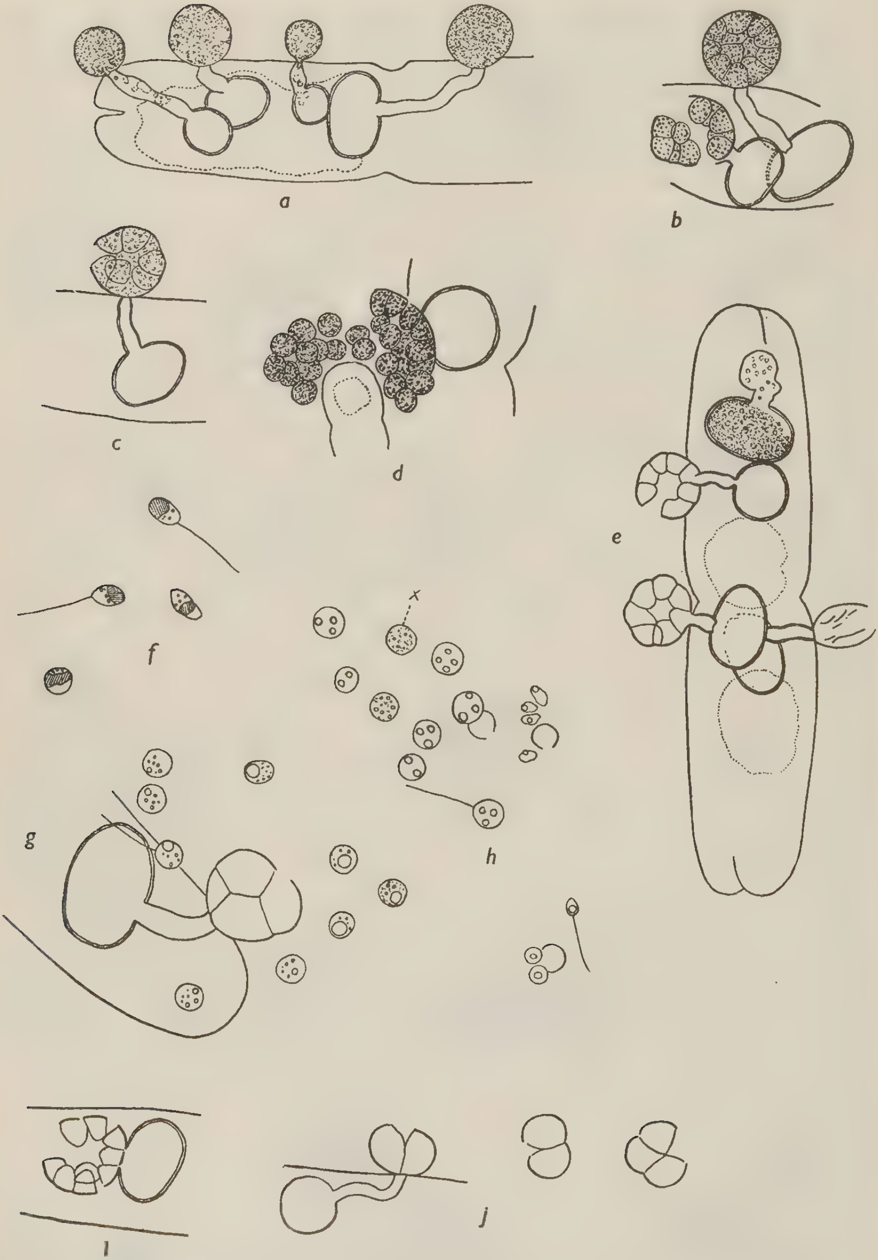
The early stages in development of the prosorus, as well as the mature prosorus resemble *Endodesmidium* and indeed, until the formation of the sorus, I believed it to be this organism. The fungus is first distinguishable as a large oil globule at one side of which granules appear to accumulate and soon a naked chytrid thallus is apparent (Text-fig. 3*a–c*), which later becomes enveloped in a smooth, colourless wall. The mature prosori are oval (12×17 to $25 \times 40\mu$) to spherical (8–12 μ in diameter); from one to fifteen (rarely thirty) occur in a single cell. Their content is densely granular (appearing brownish in the mass) and the large oil globule is usually distinguishable at one side (Text-fig. 3*d* and Pl. IX, fig. 2).

On germination, an exit tube grows from the prosorus to the outer surface of the host wall (rarely extending beyond). The content of the prosorus gradually emerges to form a spherical sorus 8–20 μ in diameter, with a smooth colourless wall. In early stages the content is hyaline (Text-fig. 3*f*, *h*), but when all the granules of the prosorus have passed out it in turn appears brown. Endobiotic sori, sessile on the prosori, were sometimes encountered. They differ in no way from the epibiotic ones, and it is considered that only one organism is present. The sorus becomes cut up into sporangia which vary in number according to its size; eight to sixteen in large sori, while small ones have only two to three (Text-fig. 4*j*). The sporangia undergo differentiation; the protoplasm becomes more oily and in each sporangium about five large bodies are formed which emerge singly through a pore to the outside medium. These bodies (primary zoospores), $4{\cdot}3 \times 2{\cdot}4\mu$, have a large anterior mass of oil, while posteriorly the cytoplasm is more granular (Text-fig. 4*f*). A single posterior flagellum is present, and although these zoospores show jerky and amoeboid movements they have never been observed swimming freely through the water. After some time they become motionless and spherical, the large mass of oil remaining clearly visible (Text-fig. 4*g*). This mass gradually becomes dispersed and the content is then more granular (Text-fig. 4*h* (*x*)). Finally from two to six spherical oil globules appear in the hyaline protoplasm, each indicating the position of a zoospore. The secondary zoospores (2 μ) emerge through a pore and swim actively through the water by means of a posterior flagellum $7{\cdot}6\mu$ long. Resting spores were not observed. This remarkable behaviour has been observed on several occasions and there is no doubt that it represents the normal course of events in this species.

Scherffel's investigations on the flagellate primary sporangial stage in *Micromycopsis cristata* Scherffel have already been referred to in connexion with *Endodesmidium* (p. 73). The organism described above lends further support to these observations. However, the number of zoospores emerging from the primary sporangia in *Micromycopsis cristata* remains unknown. The phenomenon of two sporangial and zoosporic phases is unique in the chytrids and among other aquatic fungi most nearly resembles that found in the Cystogenes group of *Allomyces* (Emerson 1941). Here the resting spores on germination produce large sluggish biflagellate zoospores which soon encyst and later give rise to four posteriorly uniflagellate zoospores. A similar condition is seen in the diplanetism of members of the Saprolegniaceae. The sporangium in *Saprolegnia* may, perhaps, be considered a sporangiosorus, each zoospore giving rise to a single sporangium (the encysted primary zoospore), which later liberates but a single zoospore.



Text-fig. 3. *Micromycopsis fischeri*. *a–c*, naked prosori. *d*, mature prosori. *e*, small spherical prosorus with large oil globule. *f*, very early stage in germination of prosorus. *g*, empty prosori with discharge tubes and sori. *h*, germinating prosori 4 p.m. 8 May 1947. *i*, *A* and *B*, 18 hours later. *j*, *A*, *B*, *C*, 42 hours later. *e*, × 1050; the rest, × 500.



Text-fig. 4. *Micromycopsis fischeri*. *a*, prosori with sori; in one content is beginning to segment. *b*, epi- and endo-biotic sorus of sporangia. *c*, sorus of sporangia, soral wall split. *d*, squashed sorus of sporangia. *e*, germinating prosorus and three empty sori. *f*, primary zoospores, anterior mass of oil cross-hatched. *g*, empty sorus with recently encysted zoospores. *h*, various stages in maturation of secondary sporangia and dehiscence of zoospores. *i*, empty endobiotic sorus. *j*, small sori with two to three sporangia. *a–e*, *i*, × 500; *f–h*, × 1050; *j*, × 800.

At present, the genus *Micromycopsis* is separated from *Micromyces* on the following characters: position of the sorus (epi- or endobiotic), nature of the soral wall (spiny or smooth), size of the sporangia, and number of zoospores produced in each sporangium. *Micromycopsis fischeri* clearly shows that these are not altogether trustworthy characters on which to base generic distinction. While the majority of the sori are formed at the end of a discharge tube as is characteristic for *Micromycopsis*, sometimes they may be sessile on the prosorus resembling *Micromyces*. Secondly, in *Micromycopsis* the sorus wall is characteristically brown and granular or spiny whereas, *M. fischeri* has the smooth colourless wall of a *Micromyces*. It is possible that many more intermediate types will be discovered, such as a *Micromyces* possessing a brown spiny sorus wall, and as suggested by Sparrow (1932) the genus *Micromycopsis* may be found to be superfluous.

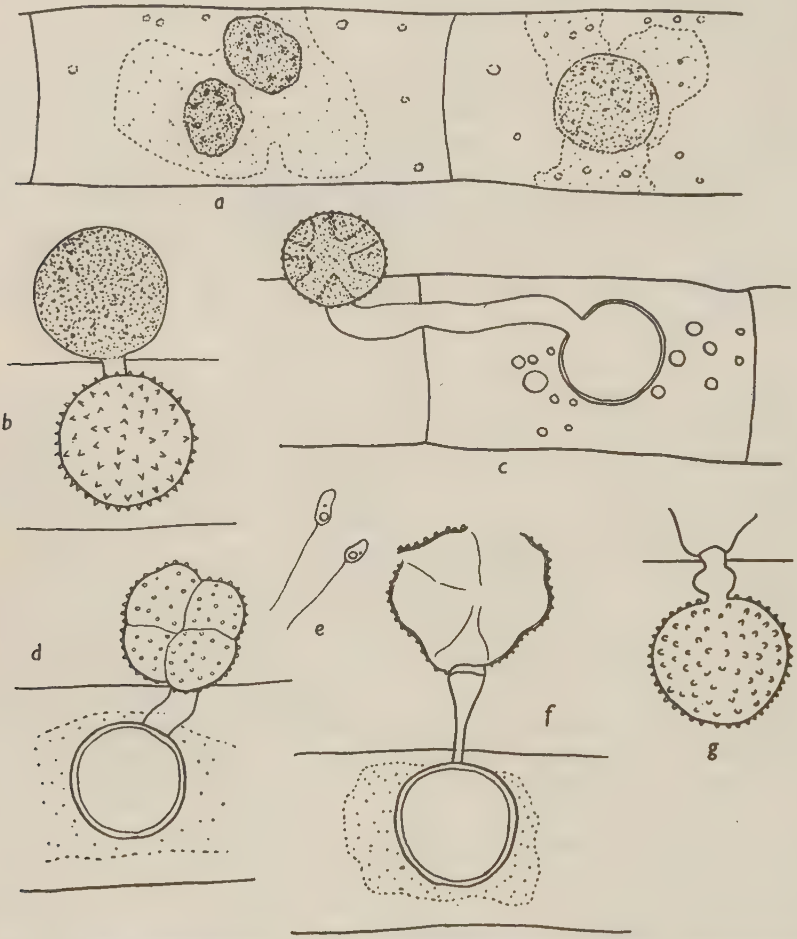
Again, early stages in the development of *M. fischeri* resemble those found in *Endodesmidium* and perhaps in the future a series of species will be found so closely linking all these genera as to make any distinction between them artificial.

III. **Micromycopsis intermedia** n.sp.

This fungus occurred on two species of *Zygnema* in the Sphagnum bog, bordering the north side of Blelham Tarn, Lancashire, during April 1946. Hypertrophy of the host cell is rarely seen, but the host chloroplast, and cytoplasm are usually reduced to a blackish mass surrounding the mature prosorus. The young thallus is first visible as a naked greyish oily mass of protoplasm, often situated near the centre of the cell (Text-fig. 5*a*). This thallus enlarges, becomes spherical, and invested by a wall to form a prosorus (10–17·8 μ in diameter), the larger specimens generally occurring singly, and the smaller in groups in the host cell. The prosoral contents are oily, and the thick brown wall may be spiny, granular or smooth (Text-fig. 5*b*, *c*). The spines are generally distributed in contrast to those of *Micromycopsis cristata* Scherffel (Text-fig. 13*a*, *b*). The prosorus on germination produces a tube which pierces the wall of the host and may extend to a varying distance beyond the host wall; at its apex, and separated by a cross-wall, a sorus develops (Text-fig. 5*f*, *g* and Pl. X, fig. 10). The spherical sorus is approximately equal in size to the prosorus and has a yellowish brown wall covered with minute blunt spines. The soral wall splits into four or six parts, exposing the same number of hyaline sporangia, each broadly triangular with a rounded base. The sporangia set free from twenty to thirty oval zoospores each with a conspicuous posterior oil globule and a smaller globule at the side. These swim actively with a single posterior flagellum. Neither reinfection of the host by the zoospores nor resting spores was seen.

Scherffel (1926) has reported that sometimes the sporangia in *M. cristata* emerge from the soral wall as relatively large uniflagellate amoeboid swarmers which then encyst and produce endogenously a few zoospores. In the species here described only the typical non-flagellate sporangial stage is known.

M. intermedia differs from all hitherto described species of the genus in that the soral wall appears to split somewhat radially into as many parts as there are sporangia. The latter are relatively few in number, broadly triangular, and liberate many zoospores. In these characters *M. intermedia* more nearly approaches the genus *Micromyces*, and in view of this it is decided to erect a new species for the chytrid here described, namely *M. intermedia.* It may also be noted that the varying position of the sorus in relation to the host wall in *M. intermedia* suggests that this is not necessarily a trustworthy specific character in the genus *Micromycopsis* (cf. *M. cristata* Scherffel, and *M. zygnaemicola* Cejp).



Text-fig. 5. *Micromycopsis intermedia* n.sp. *a*, young naked prosori in *Zygnema*. *b*, germinated spiny prosorus with sessile immature sorus. *c*, smooth-walled prosorus with long discharge tube and sessile sorus showing signs of division into sporangia. *d*, smooth-walled prosorus with sorus of four sporangia. *e*, zoospores. *f*, non-sessile dehisced sorus. *g*, prosorus with swollen exit tube. *e*, ×1300; the rest, ×1000.

Micromycopsis intermedia n.sp.

Prosorus spherical, 10·7–17·8 μ in diameter, wall brown, spiny, granular or smooth; sorus epibiotic, sessile on the host wall or at some distance from it; spherical, wall yellowish brown, covered with minute blunt spines, splitting into four to six parts to expose the same number of broadly triangular sporangia; zoospores twenty to thirty, oval (3·5 × 1·5 μ) with a conspicuous oil globule, and smaller refractive globule at the side. Resting spores unknown.

In *Zygnema* spp. Blelham Bog, Wray Castle, England.

Micromycopsis intermedia sp.nov.

Prosorus sphaericus, 10·7–17·8 μ diam., tunica brunnea, aculeata, granulosa vel laevi. Sorus epibioticus, sessilis vel pedicellatus sphaericus, tunica flavo-brunnea, aculeis minutis obtusis ornata, in 4–6 partes ita fissa ut sporangia aequinumera late triangularia monstret. Zoosporae 20–30, ovales, 3·5 × 1·5 μ, guttula oleosa distincta et guttula refractiva minore praeditae. Sporae perdurantes ignotae.

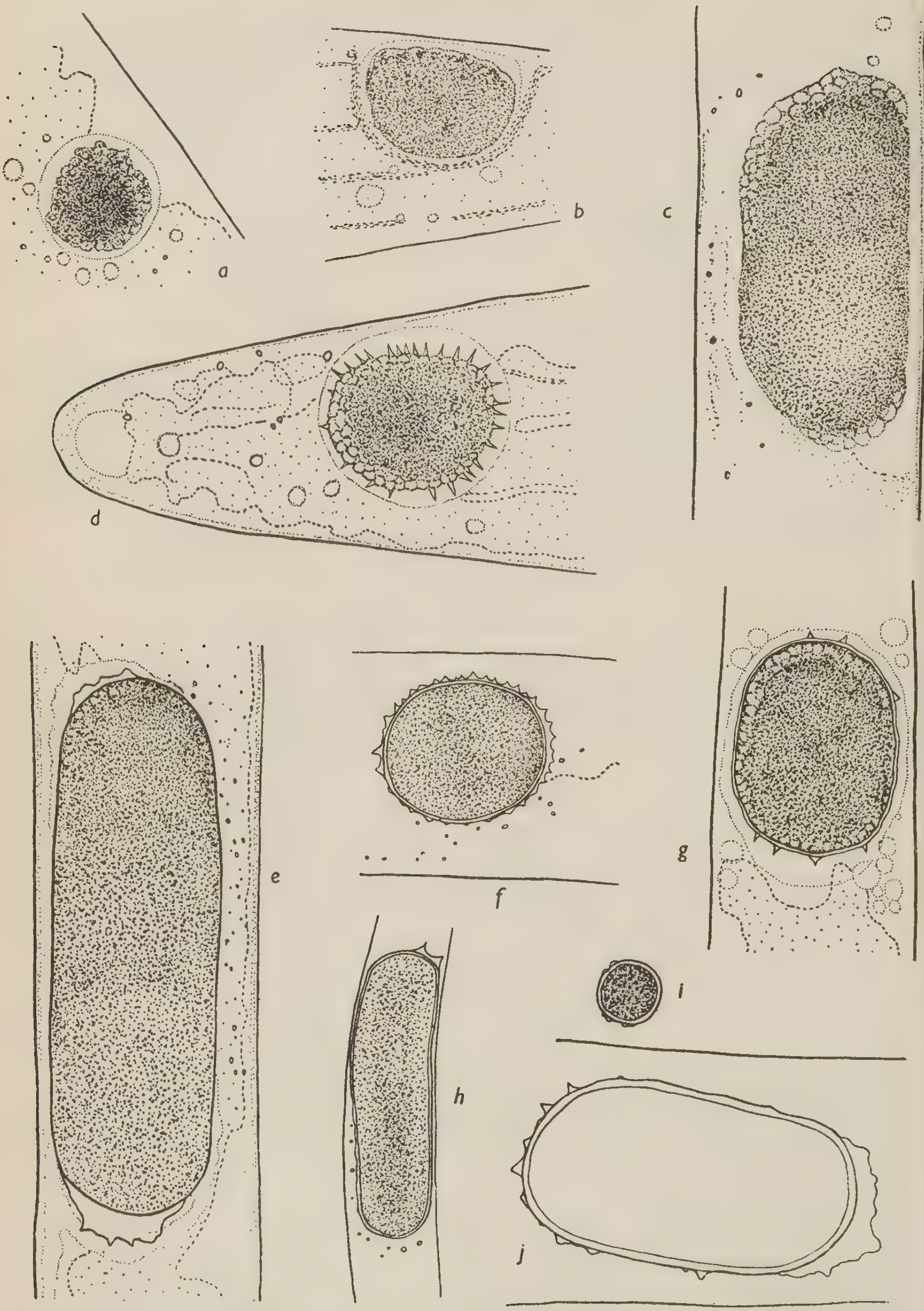
Hab. in *Zygnemate* spp., Blelham Bog, Wray Castle, Angliae.

IV. **Micromycopsis mirabilis** n.sp

This interesting species occurred in *Closterium lunula* Ehrenb., *C. dianae* Ehrenb., *C. costatum* Corda., *C. kutzingii* Bréb., and *Closterium* sp. in a collection from a sphagnum pool on the northern edge of Blelham Tarn in January 1947. Although other members of the Conjugales were present, they were not attacked.

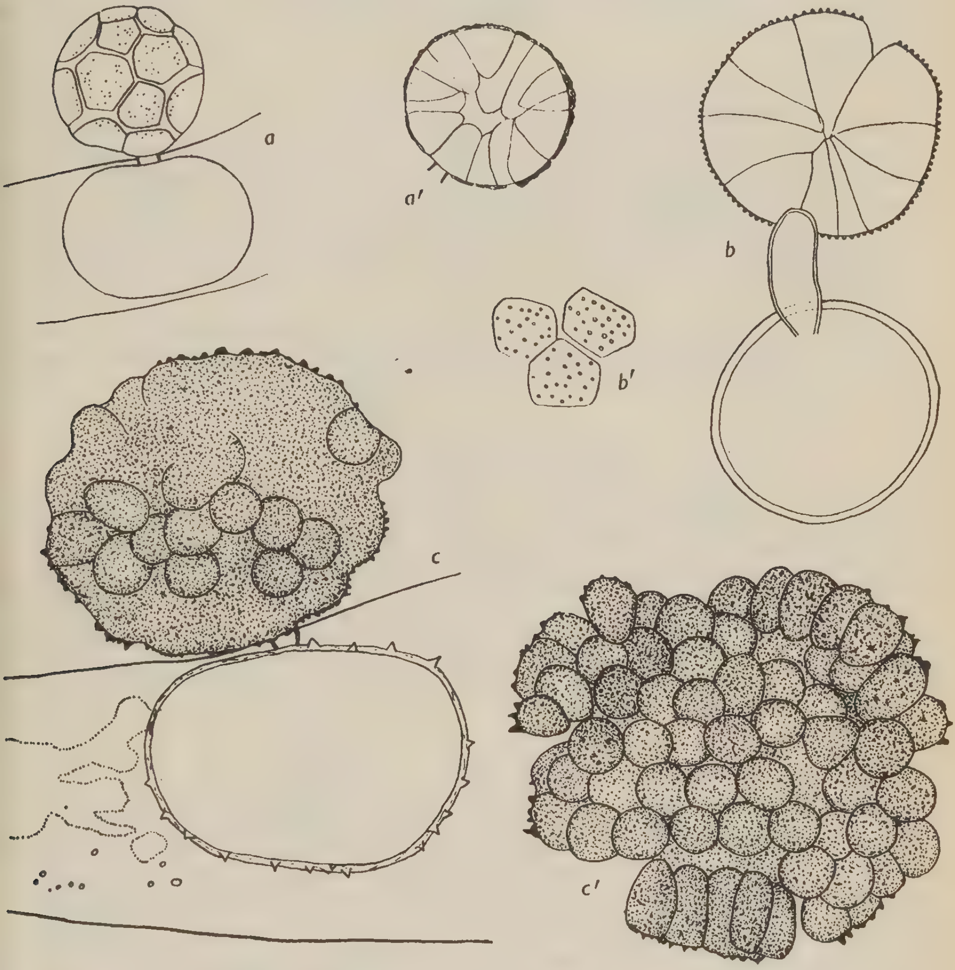
The young naked prosorus consists of a dense mass of globules, appearing black under the microscope, surrounded by a hyaline area (Text-fig. 6*a*, *b*). Before the prosorus becomes walled the spines are laid down as naked protoplasmic structures (Text-fig. 6*d*, and Pl. XI, fig. 2). The mature prosorus has dense contents giving it a blackish appearance and a hyaline wall which sometimes appears to consist of two layers. The outer bears the broad-based spines which may surround the prosorus. If, however, the latter is very elongated and the side walls are closely adpressed to the host wall (e.g. in a narrow *Closterium* sp.), then the spines may be visible only on the end walls, sometimes exhibiting an irregular form (Text-fig. 6*g*). The inner layer of the prosoral wall is smooth. In old empty prosori the spines are difficult to see and often seem to disappear completely. The prosori vary in shape and size and tend to be smaller when several occur together; seventeen have been found in a single *Closterium*. The prosori can be divided into three classes: the very elongate, 57 × 112 to 20 × 75 μ; less elongate, 55 × 67 to 12·8 × 21 μ and subspherical, 35 × 37 to 10 × 12 μ.

It may be that some of these bodies related to prosori are in actual fact resting spores. Isolated specimens, having been liberated after disintegration of the host cell, were seen in the decaying algal material and only three germinated prosori were found. The prosorus germinates by a short, thick-walled exit tube and produces a sorus immediately outside the alga.



Text-fig. 6. *Micromycopsis mirabilis* n.sp. *a–c*, naked prosori. *d*, naked prosorus with developing spines surrounded by a conspicuous hyaline area. *e–j*, mature prosori showing variation in size and degree of development of the spines. All × 500.

The sorus is spherical, roughly equal in size to the prosorus with a yellowish brown wall covered with small blunt spines, and at maturity contains ten to one hundred broadly triangular zoosporangia (Pl. XI, fig. 3). The sorus, when viewed from above, shows polygonal areas which mark the position of the zoosporangia (Text-fig. 7*a*). Infection of the *Closterium* by the zoospores was not seen, nor could any empty zoospore cases be found.



Text-fig. 7. *Micromycopsis mirabilis* n.sp. *a*, empty prosorus and sorus, outlines of zoosporangia seen in surface view. *a'*, the same in optical section. × 500. *b*, prosorus and sorus in optical section. *b'*, part of soral wall seen in surface view. × 1050. *c*, very large prosorus with immature sorus; lines of development of a few zoosporangia are visible. × 500. *c'*, the same rather flattened a day later. × 500.

In the same collection one specimen of a *Micromycopsis* sp. was observed on *Tetmemorus granulatus* (Bréb.) Ralfs. The prosorus was ornamented with granules exhibiting a somewhat spiral arrangement and the sorus possessed a yellowish spiny wall (Pl. XI, fig. 4).

Although incompletely known, these two organisms suggest that there are many forms yet to be discovered. They also, with *Micromycopsis intermedia*, indicate that the zoosporangia in this genus (hitherto in *M. cristata* and *M. zygnemicola* known to be usually spherical and to produce a few zoospores) may more closely resemble those known in *Micromyces* where they are broadly triangular, radially arranged and liberate numerous zoospores.

Micromycopsis mirabilis n.sp.

Prosorus (resting spore?) very elongate, 75 × 20 to 112 × 57 μ; less elongate, 21 × 12·8 to 67 × 55 μ or spherical, 12 × 10 to 37 × 35 μ; wall thick, hyaline, outer layer with broad-based spines. Sorus epibiotic, spherical, 28–80 μ in diameter, wall yellowish brown covered with small spines, formed at the end of an exit tube and at maturity containing ten to one hundred triangular zoosporangia 13–21 μ high × 7–14 μ broad at the base. Zoospores not observed.

Parasitic in *Closterium lunula* Ehrenb., *C. dianae* Ehrenb., *C. costatum* Corda, *C. kutzingii* Bréb., and *Closterium* sp. in a Sphagnum pool bordering the northern edge of Blelham Tarn near Wray Castle, England.

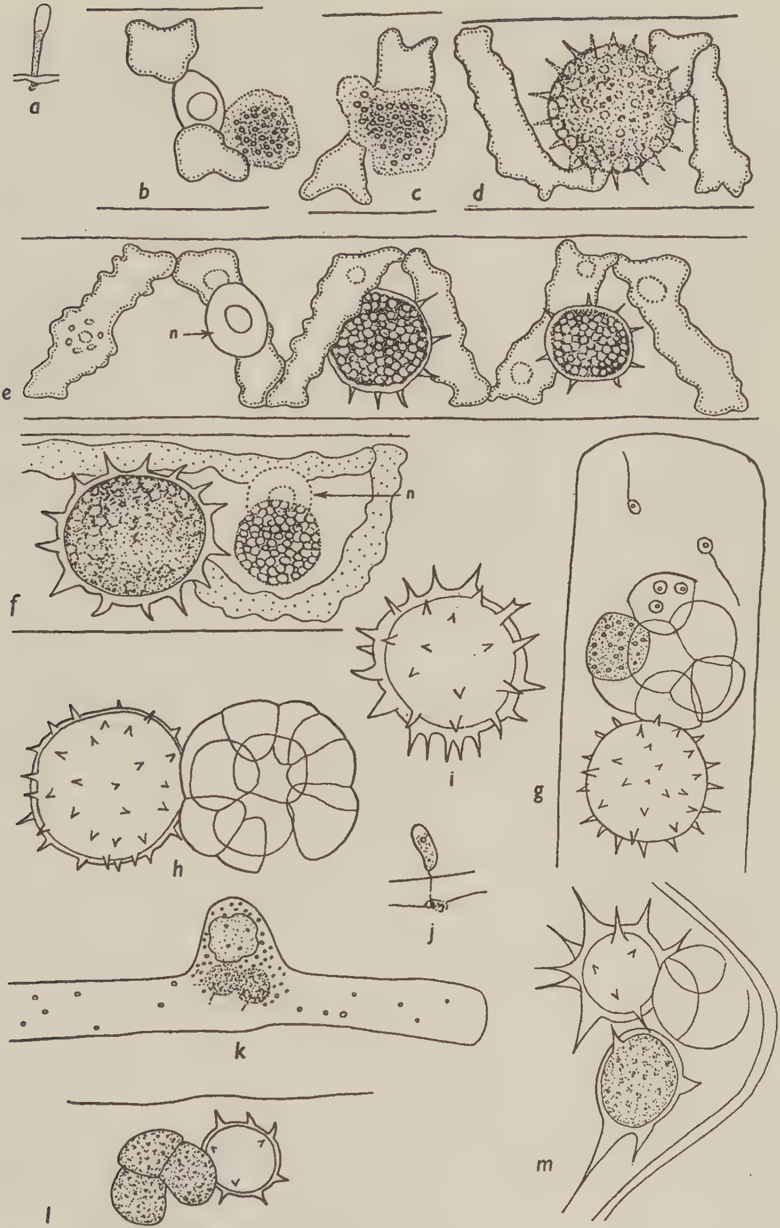
Micromycopsis mirabilis sp.nov.

Prosorus (vel spora perdurans?) praelongus 75 × 20 ad 112 × 57 μ, vel brevior 21 × 12·8 ad 67 × 55 μ, vel sub-globosus 12 × 10 ad 37 × 35 μ, tunica crassa, hyalina, aculeis basi crassis ornata praeditus. *Sorus epibioticus* globosus, 28 ad 80 μ diam., tunica flavo-brunnea, aculeis minutis ornata praeditus; in maturitate 10 ad 100 sporangia triangularia, 13–21 μ alta, basi 7–14 μ lata includens. *Zoosporae* ignotae. Hab. Parasiticus in *Closterio lunula* Ehrenb., *C. dianae* Ehrenb., *C. costato* Corda, *C. kutzingii* Bréb., et *Closterio* sp.; Blelham Tarn prope Wray Castle, Angliae.

V. *MICROMYCES ZYGOGONII* DANGEARD

Micromyces zygogonii Dangeard, described from many parts of the world, appears to be the commonest species of this genus. It occurred on *Spirogyra* sp. in the Clay Pond, Wray Castle in April 1946, but did not attack *Mougeotia* sp. and *Closterium* spp. which were also present. The zoospore encysts on the surface of the host cell, and its content passes in as a naked mass of protoplasm (Text-fig. 8*a*), leaving an empty zoospore case on the outside. This naked protoplast, either by its own amoeboid movements or by streaming of the host cytoplasm, reaches a position near the nucleus. This apparent attraction of young thalli to the host nucleus was also noted by Couch (1931, 1937). The young thallus is clearly distinguishable from the host content as a dense, greyish mass of protoplasm with numerous highly refractive globules grouped towards the centre. The peripheral region is finely granular and amoeboid, pseudopodia being constantly formed and retracted (Text-fig. 8*b*, *c*). The globules in the centre, however, do not alter their position.

The naked thallus (Pl. X, fig. 1) develops into a spiny walled prosorus. The spines appear before the wall is laid down as naked granular strands



Text-fig. 8. *Micromyces zygogonii*. *a*, contents of encysted zoospore passing into host cell. *b*, *c*, young naked amoeboid thalli. *d*, spherical naked thallus, spines beginning to develop. *e*, two mature prosori. *f*, mature prosorus and young naked prosorus adpressed to the nucleus which is represented by a dotted line. *g*, empty prosorus with a sorus of sporangia, one undehisced; two flagellate zoospores figured. *h*, empty prosorus and sorus of sporangia. *i*, thick-walled spiny resting spore. *j*, *M. petersenii* Scherffel contents of zoospore passing into *Mougeotia* cell. *k*, hypertrophied host cell with two naked prosori above which is the shrunken chloroplast. *l*, short spined prosorus with developing sorus. *m*, prosori with well-developed spines. *a*, *b*, *f*, *h*, *l*, × 1000; *c*, *d*, *g*, *m*, × 975; *i*, *j*, × 1333; *k*, × 375. *n* = host nucleus.

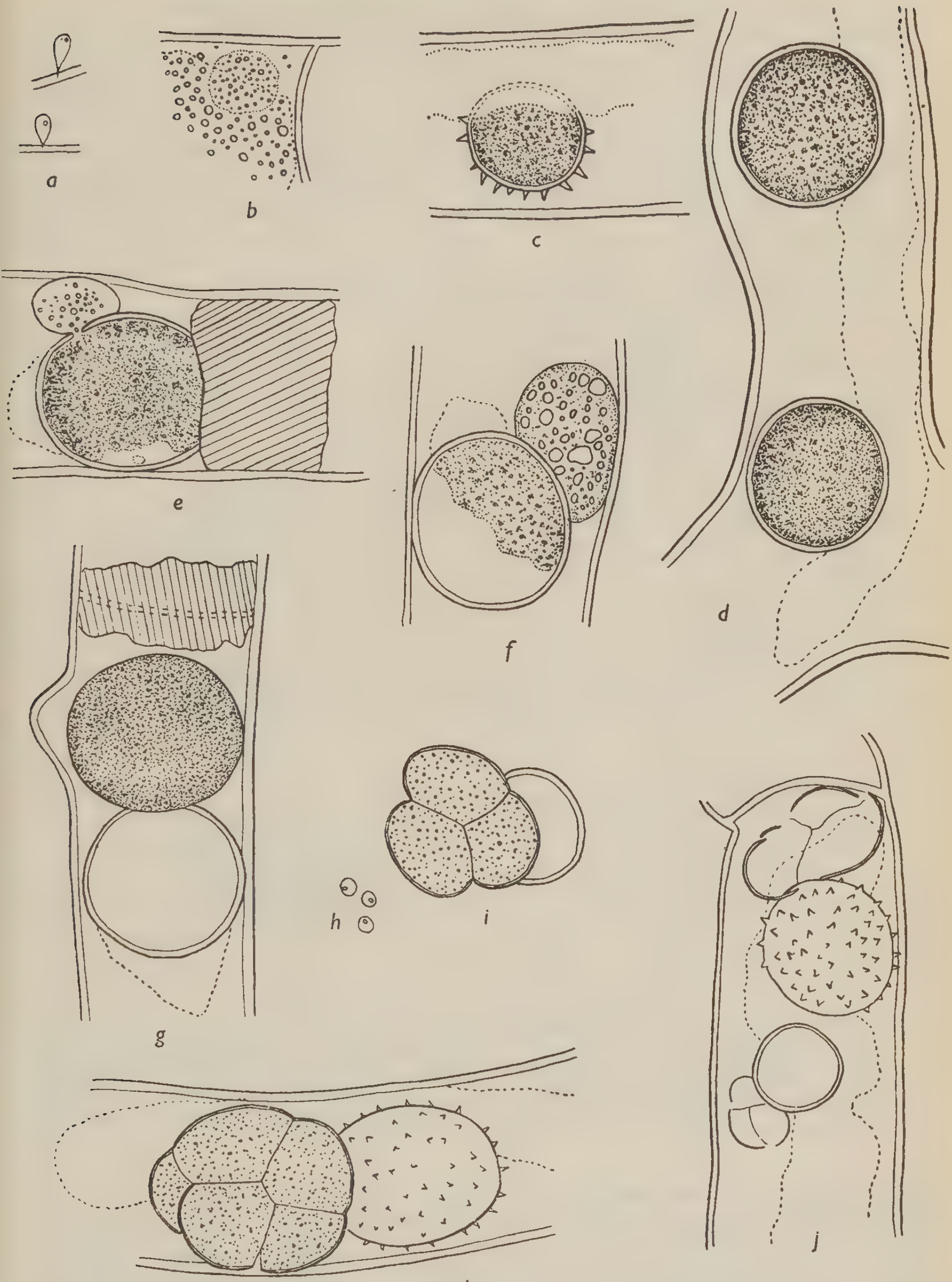
of protoplasm (Text-fig. 8*d*) which later become walled. Couch (1931) observed similar granular strands, mostly connected with the pyrenoids of the host cells, but this connexion was not observed in the material from the Lake District. The mature prosorus is spherical, 13·4–17 μ in diameter, with a colourless wall bearing numerous sharply pointed spines about 3·5 μ long (Text-fig. 8*e*, *f*). By the time the prosorus is mature the algal chloroplast shows signs of disintegration, but the distinct hypertrophy of the host cells so commonly recorded (cf. Dangeard, 1889; Huber-Pestalozzi, 1931; Heidt, 1937) was not seen. The prosorus germinates to give a spherical thin-walled sorus, 13–17 μ in diameter. Zoosporangia are delimited within the sorus and its membrane splits into about eight portions each corresponding to one zoosporangium (Text-fig. 8*g*, and Pl. X, fig. 2). The zoosporangia are 7–9 μ in diameter, broadly triangular with a rounded base each liberating about twenty zoospores. The zoospore is oval to spherical, 2 μ in diameter, with a single oil globule, and posterior flagellum.

Resting spores (Text-fig. 8*i* and Pl. X, fig. 3) appeared as the *Spirogyra* became moribund, and were often seen in empty host cells whose contents had been ingested by Protozoa. The resting spore, 11–15 μ in diameter, resembles the prosorus except for its thicker brown wall, and on germination functions as a prosorus.

Later, in January 1947, a species of *Micromyces* was found in a temporary mud pool, bordering the northern edge of Blelham Tarn Sphagnum bog, near Wray Castle, which differs in only very minor respects from *M. zygogonii*. It appears to be highly specific to one species of *Mougeotia* and does not attack *Spirogyra* and *Zygnema* spp. Hypertrophy of the host cell is not always produced, but in nearly every infected cell numerous colourless globules collect at the septa, forming a greyish mass which subsequently turns black (Text-fig. 9*g*).

The young stages in development of this form are similar to those previously described (Text-fig. 9 *a*, *b*). The mature prosori (Text-fig. 9 *c*, *d*) are spherical to subspherical, 6–22 μ diameter, with a thick hyaline, smooth or spiny wall. The contents are densely granular, and give a brownish colour to the prosorus. The smooth-walled sorus (Text-fig. 9 *g*–*k*) is approximately equal in diameter to the prosorus and at maturity the contents divide to form four to eight broadly triangular sporangia, 12–15 μ high × 8–3 μ broad, containing numerous uniguttulate, posteriorly uniflagellate zoospores, 2 μ in diameter.

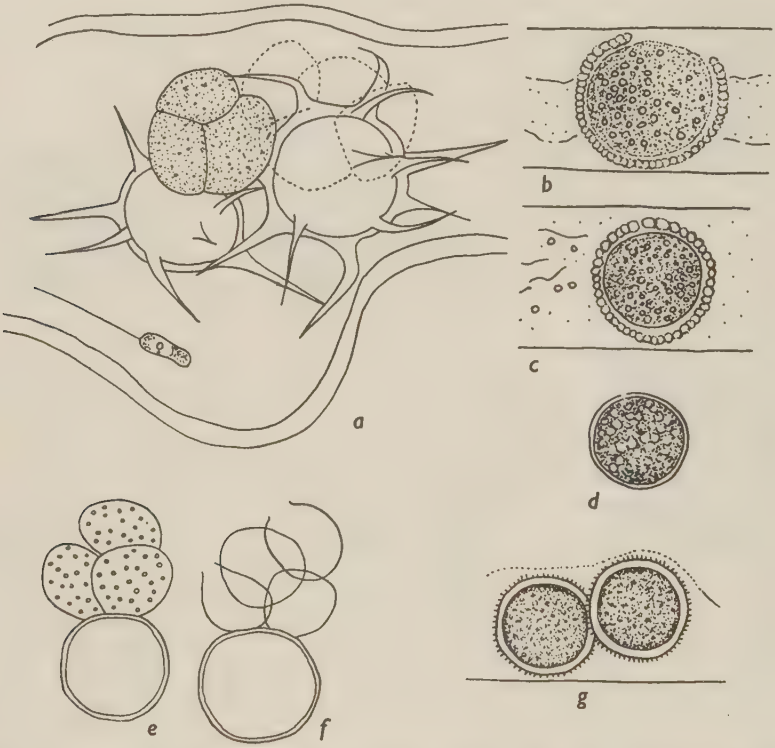
This fungus differs from *Micromyces zygogonii* only in the presence of smooth-walled as well as spiny prosori, and in the characteristic blackening of the septa of the host cell. There is as yet no clear idea of trustworthy taxonomic characters for use in specific distinction, and for the present this fungus is included in the species *M. zygogonii*. It is very probable that this is not the same fungus as occurs in *Spirogyra* in the Clay Pond, since investigations seem to indicate that the majority of aquatic Synchytriaceae are highly specific to their particular hosts. It may be found in the future that many forms with similar morphological characters can only be distinguished by physiological differences.



Text-fig. 9. *Micromyces zygogonii* Dangeard. *a*, encysted zoospores. *b*, naked prosorus. *c*, spiny walled prosorus. *d*, two smooth-walled prosori in hypertrophied host cell. *e–g*, stages in development of sorus. *h*, zoospores. *i–k*, empty prosori with immature and dehisced sori. All × 1050. Blackened region surrounding the septa in *e* and *g* cross-hatched.

VI. *MICROMYCES PETERSENII* SCHERFFEL

A form similar to *Micromyces zygogonii* was found in Blelham Bog, Wray Castle, causing hypertrophy of *Mougeotia* sp. The prosorus is spherical, 10–15 μ in diameter, with a thick colourless wall, and usually possesses a few long spines, 6–12 μ (Text-figs. 8*m*, 10*a*, and Pl. X, fig. 4); rarely a specimen was observed with short spines 2 μ long (Text-fig. 8*l*). The sorus divides into four sporangia about 7 μ in diameter which liberate from twenty to thirty zoospores. The zoospores (Text-fig. 10*a*) are oval, 5·5 × 1·5 μ, with a single oil globule, and sometimes a minute shining granule. The zoospores usually move actively, but sometimes they become amoeboid. Resting spores were not observed.



Text-fig. 10. *a*, *Micromyces petersenii* Scherffel prosori and sori inside an hypertrophied *Mougeotia* cell; one zoospore is figured. × 1333. *b*, *M. laevis* n.sp. young naked spherical prosorus with an incomplete halo of host granules in *Mougeotia* sp. × 975. *c*, almost mature prosorus with halo of granules. × 1000. *d*, mature prosorus. × 1000. *e*, germinated prosorus with sorus of sporangia; each oil globule indicates the position of a zoospore. × 975. *f*, prosorus with a sorus of empty sporangia. × 975. *g*, probable resting spores. × 1000.

This chytrid, although resembling *Micromyces zygogonii*, has zoospores of a very different size. Scherffel (1926) describes *M. petersenii* on *Mougeotia* sp. which possesses large fusiform (6 × 2 μ) zoospores, but in this species the prosorus has short conical spines sparingly dispersed over its surface. In view of the large zoospores it is decided to refer the variety here described to *Micromyces petersenii* rather than to *M. zygogonii* in spite of the difference in length of the spines.

VII. **Micromyces laevis** n.sp.

The third species of *Micromyces* was found growing on *Mougeotia* sp. in the Clay Pond, Wray Castle, in April 1946. As described for other species, the zoospore settles on the host wall, and its contents pass inside, leaving an empty case on the surface of the alga. Often beneath these empty cases there is a thickening of cell-wall material presumably stimulated by the fungal attack. The young naked thalli are usually situated near the middle of the host cell. Swelling of the latter was not seen and it was difficult to decide if the parasite caused infected cells to elongate, as they normally varied greatly in length. During the development of the thallus a partial or complete halo of colourless host granules often collects around the thallus (Text-fig. 10*b*, *c*, and Pl. X, fig. 8). Dangeard (1889, pl. 2, fig. 7) shows a similar halo in *Micromyces zygogonii*. The mature prosorus has a smooth colourless wall, oleaginous contents and varies from 13 to 18 μ in diameter (Text-fig. 10*d*, and Pl. X, fig. 7). When many prosori develop in the same host cell they tend to be smaller (7·6–11 μ). On germination a spherical smooth-walled sorus, of the same size as the prosorus, is produced. The content becomes divided to form from four to eight sporangia, and the sorus wall splits into as many parts, allowing the sporangia to separate slightly. Many oval zoospores, 1 μ in diameter, with a single oil globule and posterior flagellum, are liberated from each sporangium through an apical pore (Text-fig. 10*e*). One interesting specimen was observed in which the sorus formed outside the *Mougeotia* cell instead of endobiotically, as is characteristic for the genus *Micromyces*. Resting spores were not recognized with certainty. Thalli, 12·7 μ in diameter, similar to prosori but with a brown wall, which in three specimens appeared to be covered with very short hairs, were seen (Text-fig. 10*g*). Although they appear to germinate as readily as the normal prosori, they may, perhaps, sometimes behave as resting spores.

This species of *Micromyces* differs mainly from species already described in having a smooth-walled prosorus. Although the ornamentation of the prosoral wall appears to be so variable in species of *Micromyces*, and may prove an unsuitable character upon which to base specific distinction, it, nevertheless, seems advisable to refer this organism to a new species. *M. laevis* is suggested, taking its name from the constant, smooth-walled prosorus.

Micromyces laevis n.sp.

Prosorus spherical, 7·6–18 μ in diameter, with a smooth colourless wall; sorus endobiotic, smooth walled, spherical, with four to eight sporangia; soral wall splitting into as many parts as sporangia, zoospores numerous, 1 μ in diameter, with a single oil globule, and posterior flagellum. Resting spores, 12·7 μ in diameter, brown walled, rarely covered with numerous short fine hairs; on germination functioning as a prosorus.

In *Mougeotia* sp., Clay Pond, Wray Castle, Windermere, England.

Micromyces laevis sp.nov.

Prosorus sphaericus, 7·6–18 μ diam., tunica laeve, hyalina. Sorus endobioticus, laevis, sphaericus, sporangia 4–8 complectens, tunica in tot partes quot sporangia dissiliente. Zoosporae numerosae, 1 μ diam., guttula oleosa singula praeditae, postice uniflagellatae. Sporae perdurantes, 12·7 μ diam., tunica brunnea, rare breviter pilosa, germinatione ut prosoris se gerentes.

Hab. in *Mougeotia* sp., Clay Pond, Wray Castle, Windermere, Angliae.

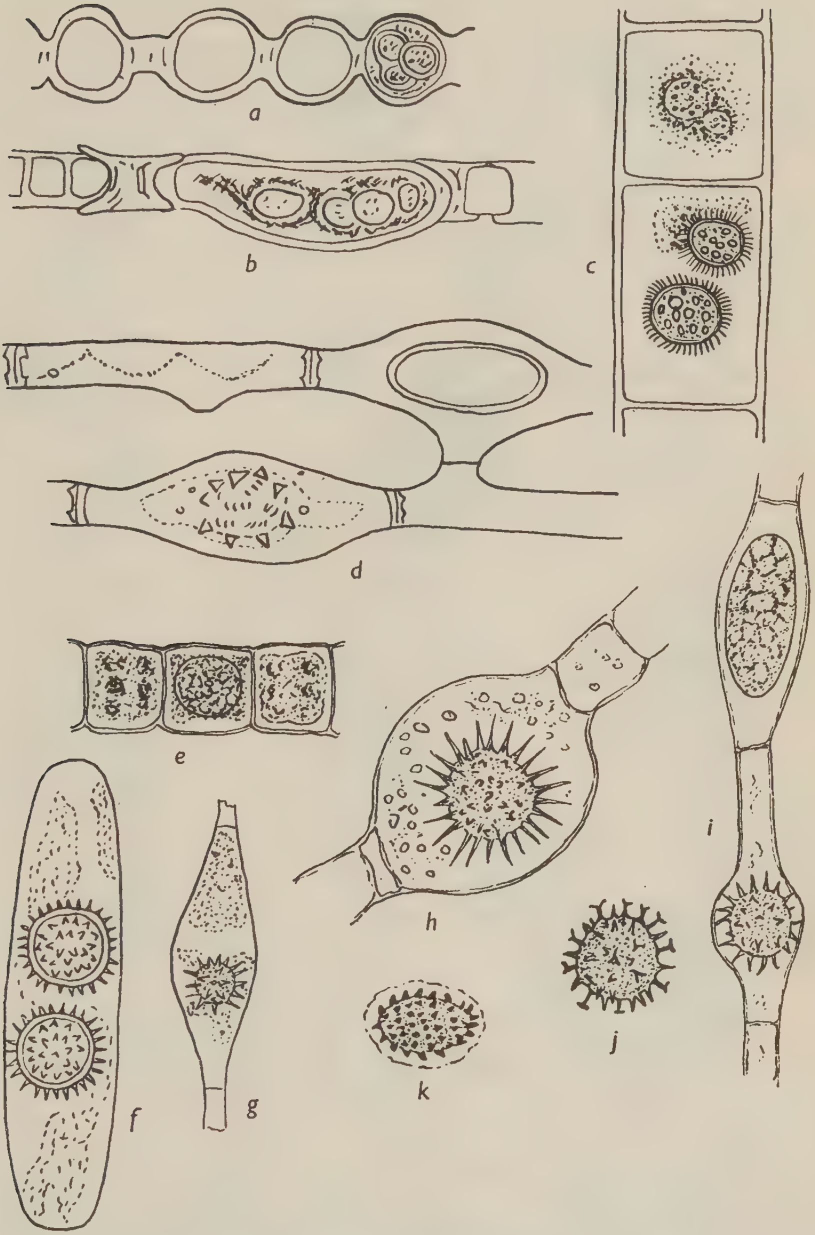
As mentioned earlier in this paper (p. 69) the older literature contains many records of spiny bodies, asterospheres, the true nature of which was then unknown, causing hypertrophy amongst members of the Conjugales. There is little doubt that some of these bodies are the prosori of species of *Micromycopsis* and *Micromyces*.

The earliest record is by Thwaites (1846–7), who found globose bodies with several long spines, causing inflation of *Mesocarpus scalaris*, and suggested that they may be an abnormal growth of the nucleus, or an internal parasite. Later, Shadbolt (1852) records spiny bodies in *Zygnema quadratum* (Text-fig. 11*i*) where sometimes the spines bifurcated (Text-fig. 11*j*), and in *Zygnema varians* with longer and more acute spines (Text-fig. 11*h*). Figures and a description are also given of an ellipsoidal body with short spines arranged on it in a regular helix in cells of the blue-green alga *Lyngbya floccosa* (Text-fig. 11*k*). Further investigations on this form would have been interesting as so far *Micromyces* and *Micromycopsis* spp. are known only as parasites of Conjugales. Other records of spiny bodies are given by Smith (1853), Pringsheim (1895), De Bary (1858) and Reinsch (1875). The parasites figured by Pringsheim (1895) in *Spirogyra* sp. appear to be of protozoan origin.

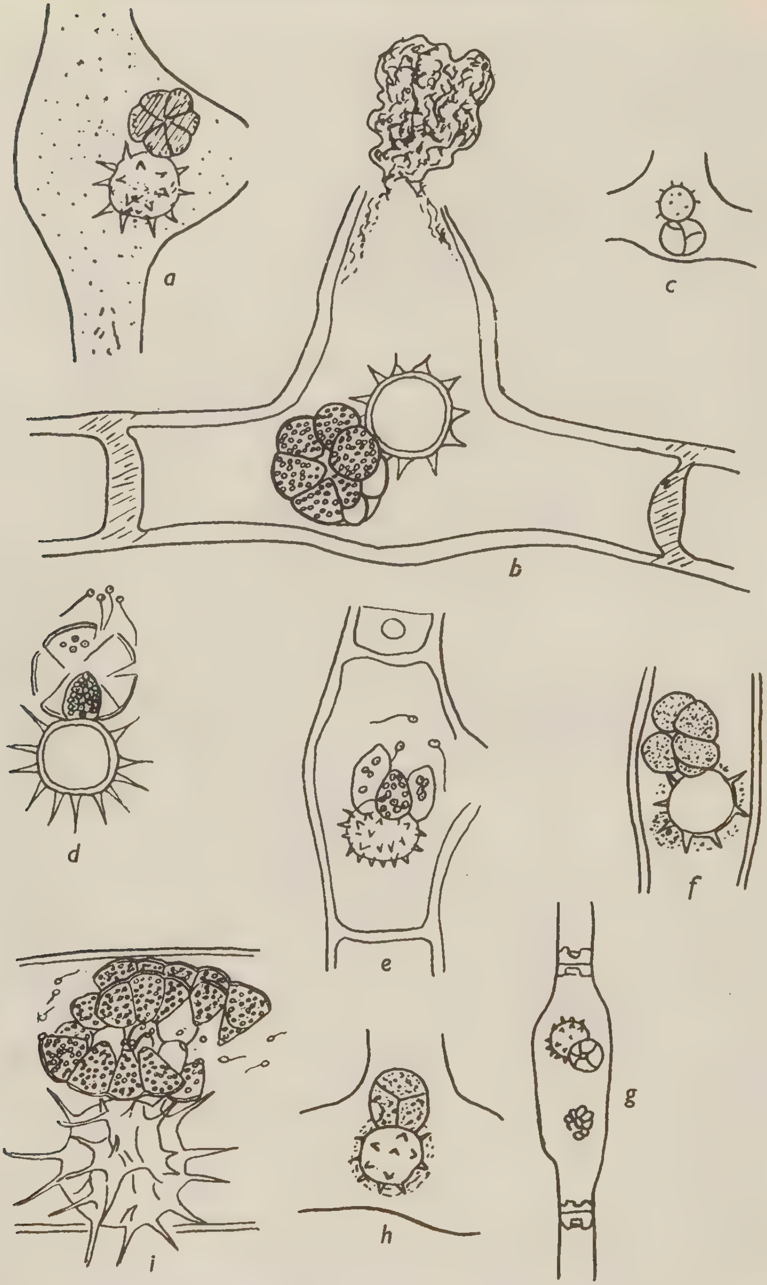
The genus *Micromyces* was erected by Dangeard in 1889 and now includes five species. Although the type species *M. zygogonii* Dangeard has many records, some must remain doubtful as germination of the prosorus has not been observed. Records where germination is known are as follows:

On *Zygogonium* sp. Dangeard (1889), *Spirogyra quadrata* Denis (1926), France; *Mougeotia* sp. Petersen (1910), Denmark; *Mesocarpus scalaris* Minden (1915), *Mougeotia scalaris* Heidt (1937), Germany; *Mougeotia* sp. Huber-Pestalozzi (1931), Switzerland; *Mougeotia* sp., *Zygogonium* sp. Couch (1937), *Mougeotia* sp. Sparrow (1943), United States.

Smooth-walled prosori causing great swelling of *Zygogonium* cells (Text-fig. 11*a*, *b*) were found by De Wildeman (1891) and referred to *Micromyces zygogonii*. Similar bodies were found by the author in *Zygogonium* sp. from Rusland Bog, Lancashire, in July 1946. In neither instance has the germination of the prosorus been observed, and therefore De Wildeman's record still remains in doubt. Other incomplete records are given by Schulz (1922; fig. 91) in *Netrium* sp. (Text-fig. 11*f*), and also (1923, figs. 10–11: cited from Sparrow, 1943) in *Mougeotia* sp. Again Denis (1926) figures an indeterminate body with a characteristic ornamentation in *Spirogyra tenuissima* (Text-fig. 11*d*). The surface of the wall is thick, finely and regularly plaited; in addition there are hyaline ridges, more or less sharp, with or without marginal teeth. Other stages are unknown.

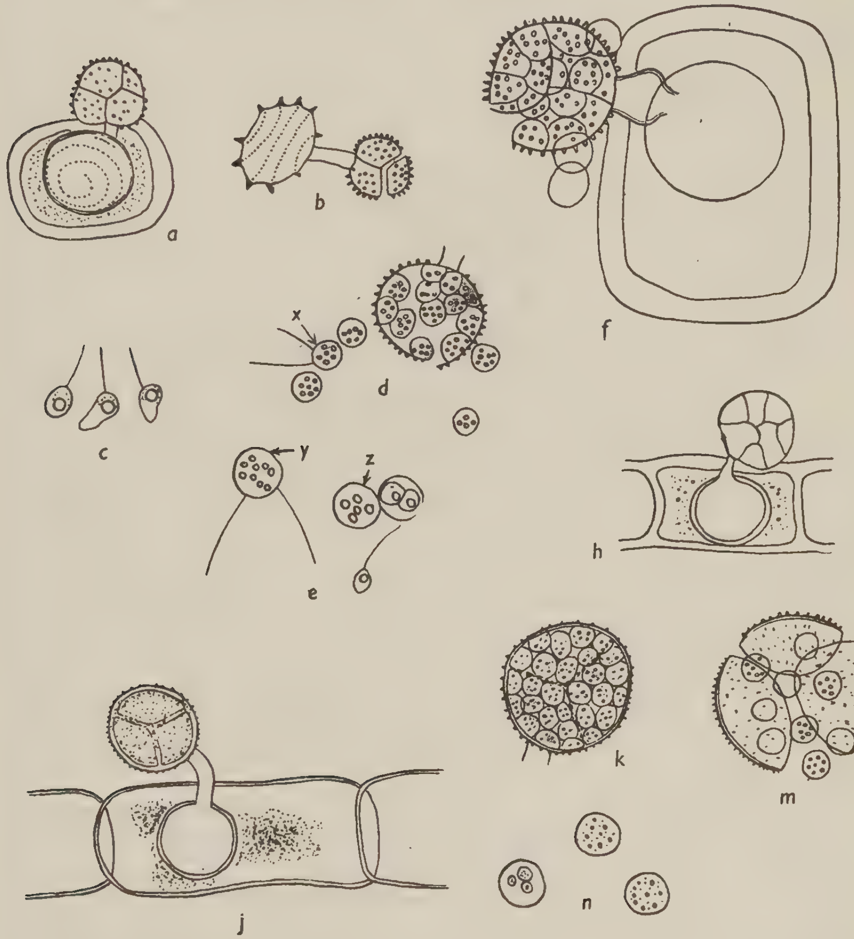


Text-fig. 11. *a*, *b*, smooth-walled prosori of *Micromyces zygogonii* in *Zygogonium* sp. (after de Wildeman, 1891). *c*, two prosori of *Micromyces spirogyrae* in *Spirogyra inflata* (after Skvortzow, 1925). *d*, unidentified internal parasitic body in *Spirogyra tenuissima* (after Denis, 1926). *e*, prosorus of *Micromycopsis cristata* in *Hyalotheca dissiliens* (after Cejp, 1933). *f*, spiny bodies in *Netrium* sp. (after Schulz, 1922). *g*, asteridia in *Mesocarpus scalaris* (after Smith, 1853). *h*, asteridia in *Zygnema varians*; *i*, *j*, in *Zygnema quadratum*, *j*, specimen with forked spines. *k*, asteridia in *Lyngbya floccosa* (*h–k* after Shadbolt, 1852).



Text-fig. 12. *a*, *Micromyces zygogonii* Dangeard, germinated prosorus with sorus of sporangia in *Mougeotia* sp. (after Huber-Pestalozzi, 1931). *b*, *Micromyces zygogonii*, prosorus with sorus of sporangia in *Mougeotia* sp. (after Heidt, 1937). ×620. *c*, *Micromyces zygogonii* (after Petersen, 1910). *d*, *e*, *M. zygogonii* in *Zygogonium* (after Dangeard 1889). *f*, *M. zygogonii* (after Couch, 1937). *g*, *M. zygogonii* in *Spirogyra quadrata* (after Denis, 1926). *h*, *Micromyces petersenii* Scherffel in *Mougeotia* sp. (after Scherffel 1926). *i*, *Micromyces longispinosus* Couch (after Couch, 1937).

For a form similar to *Micromyces zygogonii*, but differing in the larger size of the zoospores, 6 × 2 μ rather than 1 μ diameter, Scherffel (1926) erected the second species of the genus, *M. petersenii*. The only other species which has been adequately studied is *M. longispinosus* Couch (1937) (Text-fig. 12*i*). *M. spirogyrae* Skvortzow (1925) (Text-fig. 11*c*) is of uncertain affinities, as neither the germination of the prosorus, nor the structure of the zoospores



Text-fig. 13. *a–f*, *Micromycopsis cristata*. *a*, empty intramatrical prosorus with spine spirals in polar view and extramatrical sorus. × 460. *b*, as (*a*), spine rows of prosorus seen from the side. × 460. *c*, non-swarming swarmers. × 680. *d*, rounded sporangia of the spherical sorus, which probably arose from the non-swarming swarmers, in part inside, in part outside the sorus membrane; by (*x*) one has developed two fine thread-like processes. × 680. *e*, (*y*) sporangium with two processes; (*z*) without thread-like processes; nearby a discharged swarmer. *f*, smooth-walled prosorus with sporangiosorus (after Scherffel, 1926). *h*, *Micromycopsis fischeri*, smooth-walled intramatrical empty prosorus in *Zygogonium*; extramatrical sporangiosorus with a network of empty zoosporangia. × 680 (after Scherffel, 1926). *j–n*, *Micromycopsis zygnaemicola*. *j*, sporangiosorus with exit tube and empty initial stage. *k*, sporangiosorus. *m*, sporangiosorus torn into three parts with sporangia escaping. *n*, sporangia, one with three spores (after Cejp, 1932).

have been observed. Lastly, the *M. mesocarpi* of De Wildeman (1900) differs in forming an epibiotic sorus, which suggests that it more closely resembles members of the genus *Micromycopsis* as at present defined. Three species of *Micromycopsis* Scherffel have been described, namely, *M. cristata* and *M. fischeri* (Scherffel, 1926), and *M. zygnaemicola* (Cejp, 1932). *M. cristata* (Text-fig. 13*a–f*), found in *Hyalotheca dubia* from Hungary, has only one other record, by Cejp (1933) from Czechoslovakia, growing on *H. dissiliens* (Text-fig. 11*e*). However, germination of the prosorus was not observed, and this organism may equally belong to the genus *Micromyces*. A variety *M. cristata* var. *minor* was erected by Sparrow (1932) for a small form bearing sharp instead of blunt spines on the soral wall. The second species, *M. zygnaemicola* Cejp, differs from the above in having a smooth rather than spiny prosoral wall, and in the sorus being formed away from the host cell instead of on its surface (Text-fig. 13*j*). The incompletely known *M. fischeri* Scherffel differs from the other species in its smooth-walled colourless sorus which is divided into sporangia by radially arranged sutures (Text-fig. 13*h*, and see p. 74).

In view of the further studies on these organisms it has become increasingly difficult to find trustworthy characters on which to base generic and often specific distinction, and it is clear that in the near future *Micromyces* and *Micromycopsis* may have to be merged into one genus. It is also evident that Scherffel's observations (although not continuous) on the rare occurrence of a flagellate sporangial stage in *M. cristata* were probably correct. Such a phase is normally present in *M. fischeri* and *Endodesmidium formosum*. The latter may be regarded as the most primitive type, the primary non-swarming swarmers being formed separately. *Micromycopsis fischeri*, however, differs from *Endodesmidium* in the sorus being divided into sporangia each of which liberates five or more of these primary zoospores, which after liberation behave in a similar manner. Passing to *Micromycopsis cristata* this flagellate sporangial stage is rarely recorded and more typically the behaviour is as in *M. zygnaemicola* and *Micromyces* spp., where there is apparently only one sporangial stage liberating the normal chytridiaceous zoospores, which correspond to the secondary zoospores of the former types.

It thus appears that during the course of development of these organisms the primary non-swarming zoospore stage and the subsequent formation of secondary sporangia is suppressed and we get, as in most species of *Synchytrium*, a sorus of sporangia immediately giving rise to chytridiaceous zoospores. *S. fulgens* Schröter may be considered as representing the culmination of suppression in which the sorus of sporangia is formed within the prosorus.

SUMMARY

The morphology and life history of seven aquatic Synchytriaceae are described. *Micromyces zygogonii* Scherffel, *M. petersenii* Scherffel and *Micromycopsis fischeri* Scherffel are new records for Great Britain; *Micromyces laevis*, *Micromycopsis intermedia* and *M. mirabilis* are new species; and *Endodesmidium formosum* gen.nov. sp.nov.

In *Endodesmidium* the sporangia emerge from the sorus as non-swarming

zoospores, and it is believed this represents a primitive condition within the group.

References to 'asterospheres' in the older British literature are cited, and the validity of records of *Micromycopsis* and *Micromyces* spp. are considered.

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EXPLANATION OF PLATES

Endodesmidium formosum n.gen.

Plate VII

Fig. 1. Healthy cell of *Netrium oblongum* (De Bary) Lütkem. × 680.
Fig. 2. Young parasite in oil globule stage at (*a*); host contents beginning to degenerate. × 660.
Fig. 3. Two naked granular prosori. × 640.
Fig. 4. Stained mature prosorus; (*b*), more densely staining area, possibly the nucleus. (*c*) vacuole once occupied by the oil globule. × 600.
Fig. 5. Walled prosorus in *Cylindrocystis*, (*d*), anterior conspicuous oil globule. × 840.
Fig. 6. Naked prosorus in *Cylindrocystis*. × 650.
Fig. 7. Germinated prosorus with almost mature sorus in *Netrium*. × 700.
Fig. 8. Dehisced sorus; a few sporangia have failed to escape. × 660.

Plate VIII

Fig. 1. A *Netrium* cell containing three empty prosori of *Endodesmidium formosum* n.sp. with their respective sori; the latter are out of focus. The sporangia are clearly visible and that at (*x*) has two oil globules delimited, each indicating the position of a zoospore. × 1150.

Plate IX. *Micromycopsis fischeri* Scherffel

Fig. 1. Naked prosorus in *Tetmemorus Brebissonii*; the host content is little disorganized. × 860.
Fig. 2. Mature prosori. × 840.
Fig. 3. Early stage in development of the sorus. × 830.

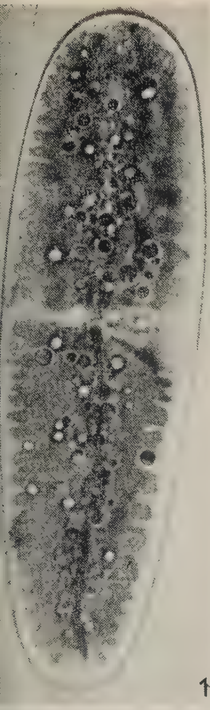
Plate X

Fig. 1. *Micromyces zygogonii* Dangeard, naked prosorus in *Spirogyra* sp.; note absence of swelling of the host cell. × 880.
Fig. 2. *M. zygogonii*, germinated spiny prosorus with dehisced sorus; one sporangium still retains its zoospores. × 860.
Fig. 3. *M. zygogonii*, brown-walled resting spore. × 800.
Fig. 4. *M. petersenii* Scherffel, empty prosorus with a few long spines in *Mougeotia* sp. × 600.
Fig. 5. *M. petersenii*, young naked prosorus. × 650.
Fig. 6. *M. laevis* n.sp., two young prosori in *Mougeotia* sp. × 630.
Fig. 7. *M. laevis*, mature prosorus. × 570.
Fig. 8. *M. laevis*, almost mature prosorus with a halo of host globules. × 600.
Fig. 9. *Micromycopsis intermedia* n.sp., sorus with five dehisced sporangia viewed from above. × 740.
Fig. 10. *M. intermedia*, two prosori in *Zygnema* sp.; one has formed an exit tube and extramatrical sorus. × 920.

Plate XI

Fig. 1. *Micromycopsis mirabilis*, part of a *Closterium* cell with eight prosori. × 430.
Fig. 2. Immature prosorus with developing spines. × 650.
Fig. 3. Empty prosorus with sorus of sporangia. × 430.
Fig. 4. Unidentified *Micromycopsis* sp. × 705.

(*Accepted for publication* 28 *August* 1947)



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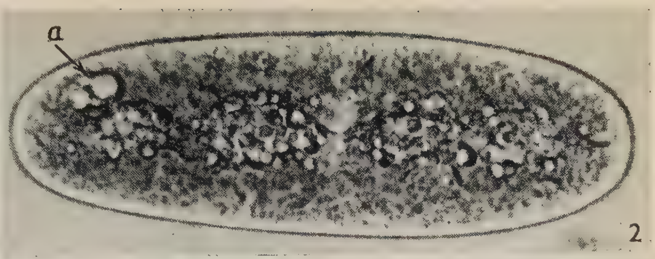

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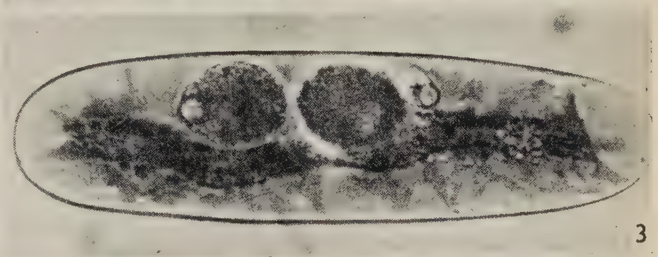

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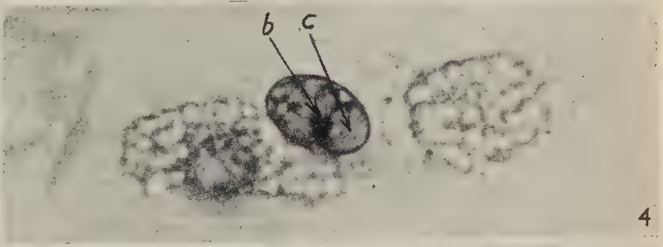

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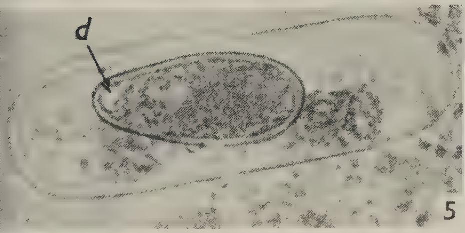

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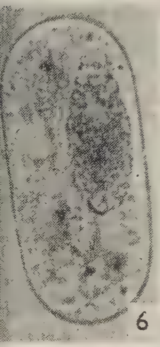

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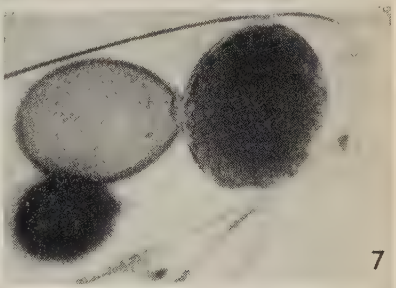

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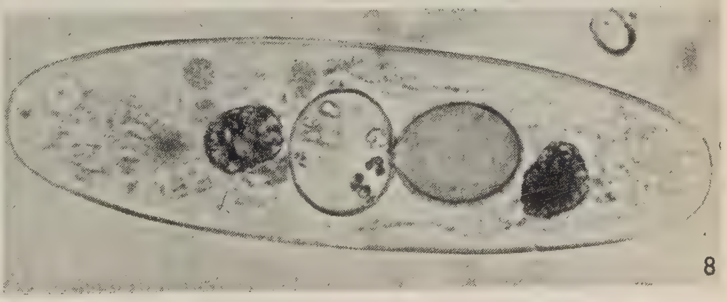

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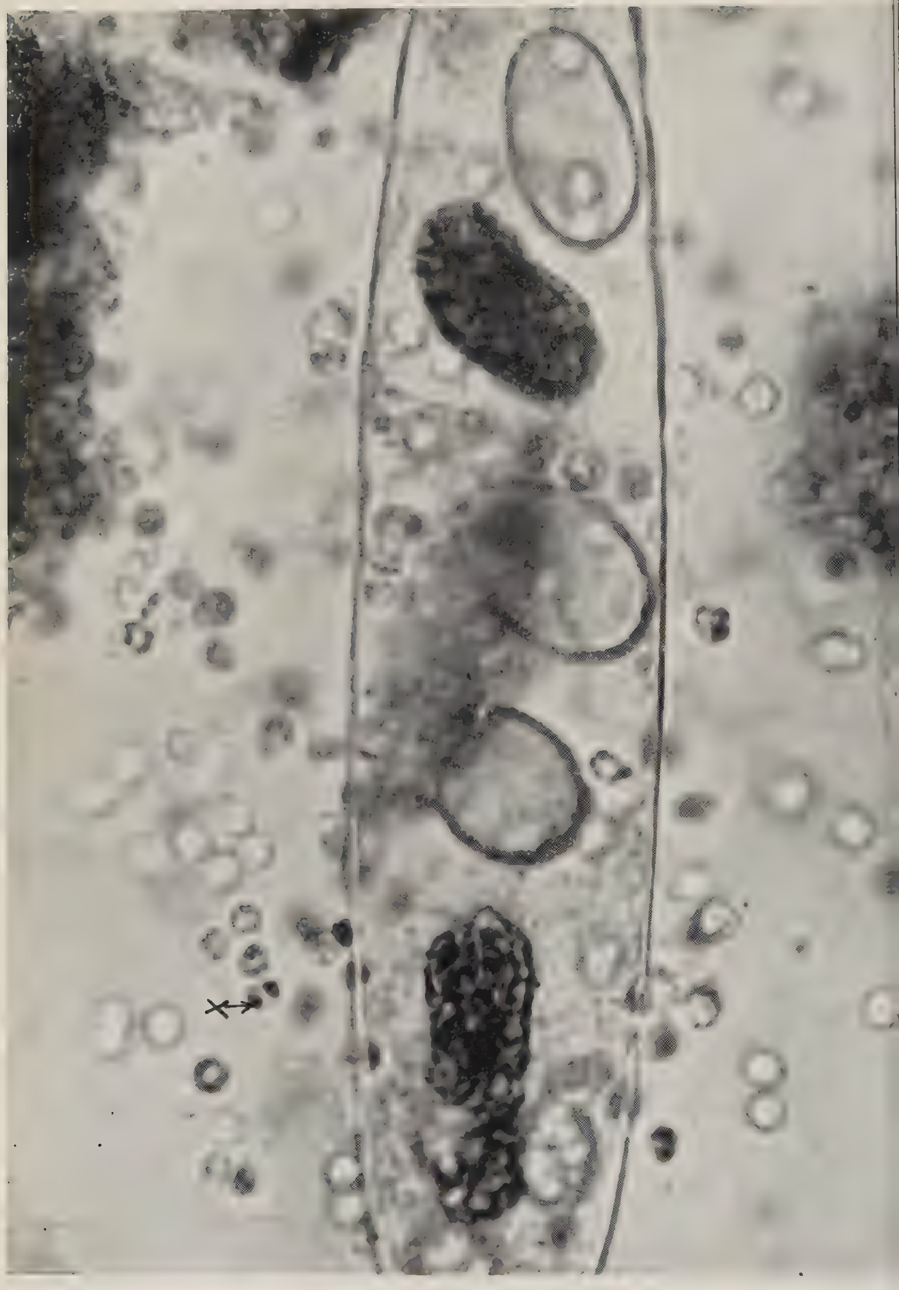
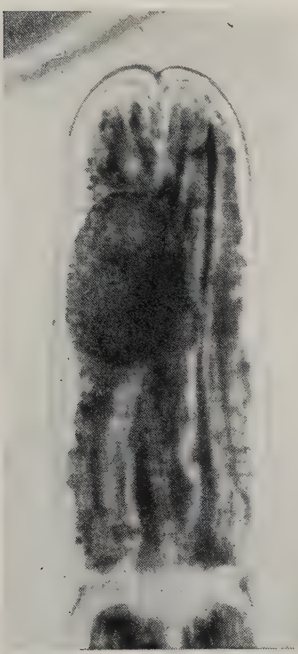


Fig. 1



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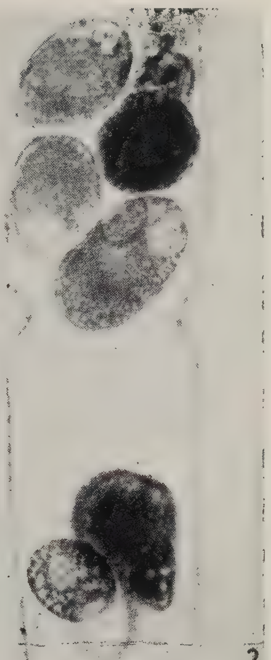

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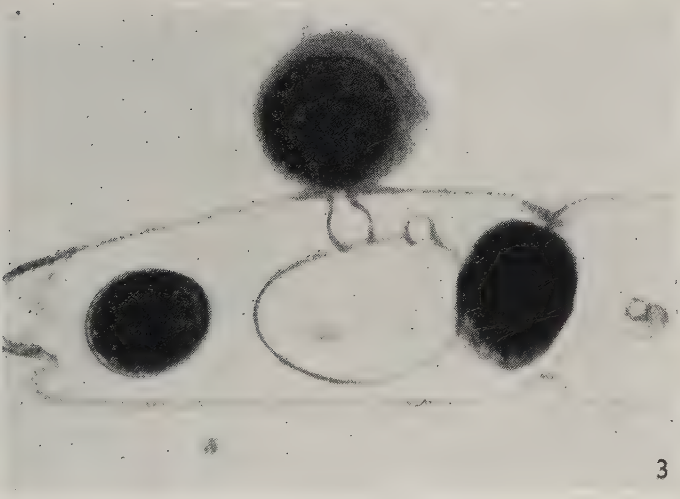

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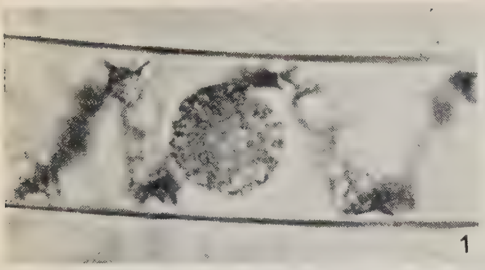

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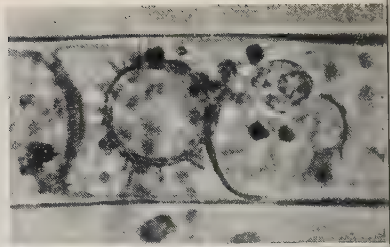

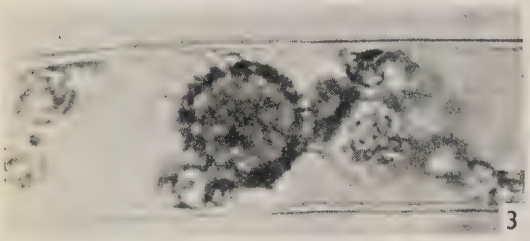

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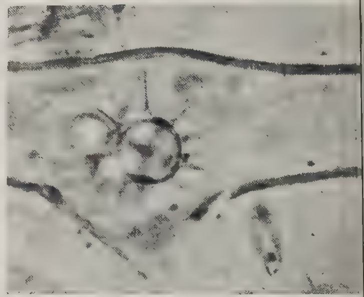

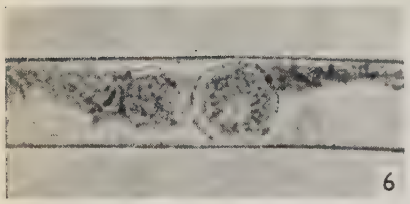

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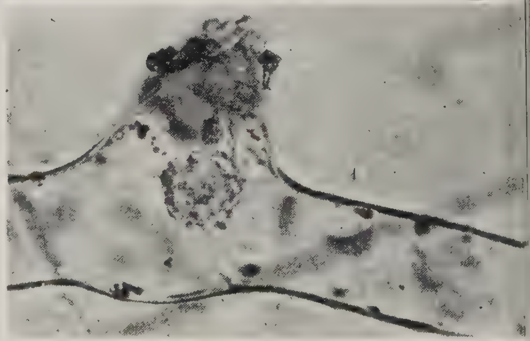

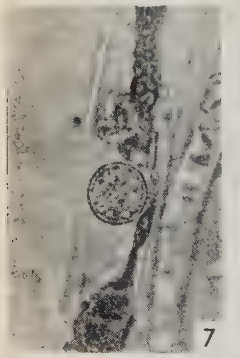

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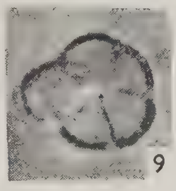

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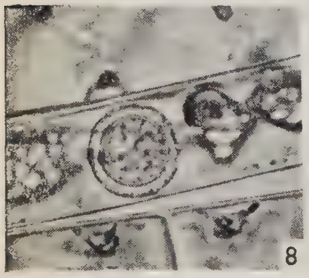

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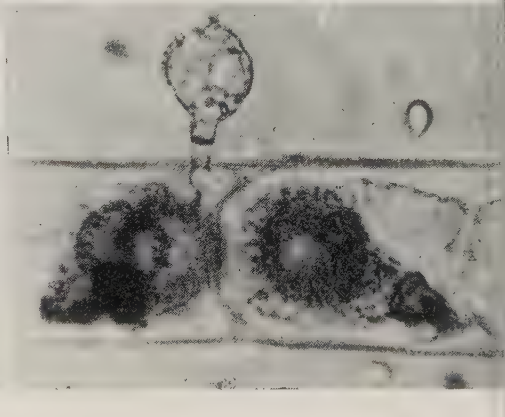

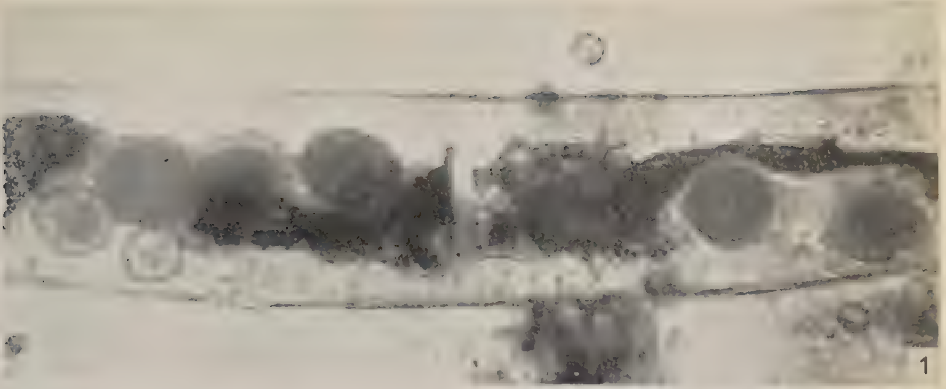
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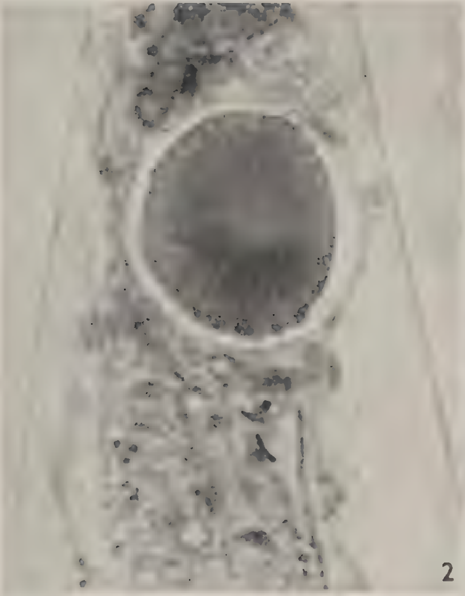
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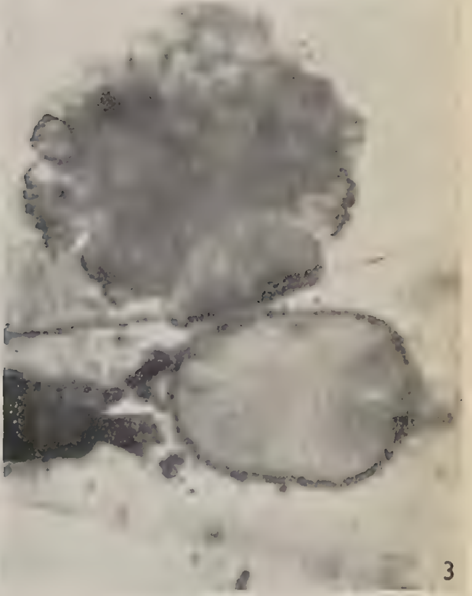
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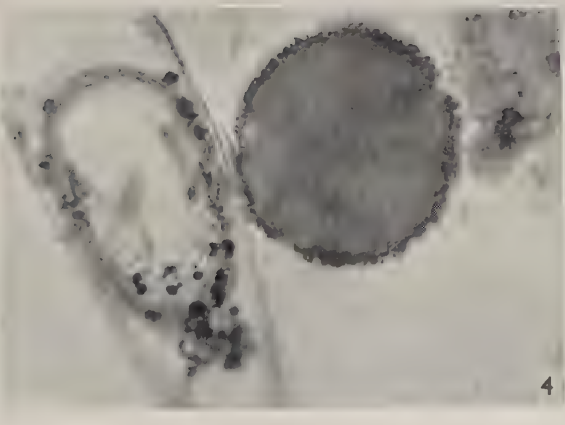
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NEW AND INTERESTING PLANT DISEASES

By W. C. MOORE, *Plant Pathology Laboratory, Harpenden, Herts*

33. Leaf blotch of *Geum* (*Zythia fragariae* Laibach)

Early in September 1941 I noticed that a number of plants of *Geum* × *Borisii*, growing in a nursery bed at Harpenden, showed dark olive-brown blotches on the leaves. The blotches were visible on both sides of the leaves, irregular in shape, scattered or spreading in from the margins in V-shaped fashion, and they occasionally covered the whole leaf. Numerous pycnidia were present on the blotches: they were mainly epiphyllous, occasionally hypophyllous, immersed then erumpent, subglobose, pale brown, thin-walled, 130–190 μ (–300 μ) in diameter. The pycnospores were 5–7 × 1·5–3 μ, hyaline, short rod-shaped, with rounded ends and a prominent guttule at each end.

The fungus was tentatively identified as *Phyllosticta gei* Bres., which has been listed by Grove (*Coelomycetes*, i, 39) on *Geum urbanum* and *G. rivale* in Ayrshire. I have examined a slide, prepared from type material of this species (on *G. urbanum*, Konigstein, 29 May 1897) kindly sent to me by Miss E. M. Wakefield, and the Harpenden material appears to me to be identical with it. The fungus, however, is not a good *Phyllosticta*: it is a species of *Zythia*, and I can see no difference between it and *Z. fragariae* Laibach, which has been found on strawberry leaves in several parts of England and Wales (Moore, 1943; Wormald, 1944). Moreover, Dr Wormald has told me that he succeeded in infecting leaves of *Geum* with *Zythia* from strawberry.

Phyllosticta gei Bres. (*Hedwigia*, 1900, 325) is antedated by *P. gei* Thüm. (*Bull. Soc. Imp. Nat. Moscou*, LVI, Part 2, 1881, 130) which, judged from its description, is a different fungus, with pycnidia 'opaco-nigricis', and therefore probably a true *Phyllosticta*. It would seem that two species are passing current under the name *P. gei*, and confirmation of this was given me in a letter received from Dr I. Jørstad, who informed me that the *Phyllosticta*, commonly occurring in Norway on *Geum rivale* and *G. urbanum* does not fit *Phyllosticta gei* Bres. but is clearly identical with Jaap's fungus on *Geum* referred to by Diedicke in *Krypt. fl. Mark Brandenb.* IX, Pilz, vii, 55 (1912). As *P. gei* Bres. is a homonym and therefore invalid, *Zythia fragariae* Laibach is a valid name for the fungus that causes leaf blotch of *Geum* and strawberry. Its perfect state, according to Wormald (1944), is a species of *Gnomonia*, possibly identical with *G. herbicola* A. L. Smith (1910).

34. Downy Mildew on *Alyssum saxatile* (*Peronospora galligena* Blumer)

On 11 November 1946 Mr W. Buddin sent me some leaves and young seedlings of *Alyssum saxatile* affected with a Downy Mildew. They had been sent to him from a nursery at Slough (Bucks), and according to particulars which he later obtained, the affected plants had been raised from seed sown rather thickly in shaded beds under glass in May. The mildew was first noticed, probably in July, and dusting with sulphur failed to check it. Though previously thinned, the seedlings were rather leggy when transplanting was carried out on 7 November.

The affected leaves showed what appeared to be small yellowish or brownish green galls or blisters, about two or three millimetres across and sometimes as high, on the upper surface. Actually, however, these small areas were 'humps' on the upper surface where the leaf tissues were locally puckered, and corresponding 'hollows' were seen on the lower surface when the leaf was turned over. Occasionally the humps appeared on the lower surface and the midribs below were locally swollen. The leaves had a yellowish cast and a number of them were deformed, especially when the midrib was swollen. Conidiophores of a *Peronospora* were present on both sides of the leaves, but were particularly abundant on the underside. For the most part they developed in small tufts on or just around the puckered spots, and the hollows on the underside were usually filled with a white mass of mycelium and conidiophores. There were, however, isolated conidiophores, or tufts of them, on those parts of the leaves which showed no obvious sign of puckering, and especially on the under surface. The conidiophores were up to 1·2 mm. long, rather lax, unseptate, 9–12 μ broad, and unbranched except at the distal end where there were a few main branches that divided repeatedly to give numerous ultimate branchlets, tapering to a width of 2 μ and ending in curved prongs with rounded tips, each bearing a conidium. The conidia were mostly broadly egg-shaped, occasionally spherical, 15–24 × 14–19 μ (average of twenty-five conidia, 18 × 15 μ), with thin smooth walls and granular contents.

The fungus was clearly identical with *Peronospora galligena* Blumer, which Blumer (1938) described as common around Berne in Switzerland on *Alyssum saxatile*, and as occurring in the Botanic Gardens at Berne on its varieties *citrinum* and *compactum*. The same species was reported on *A. saxatile* soon afterwards in various parts of Germany, including the Rhineland (Laubert, 1938) and the Dresden area (Pohlig, 1939), as well as on var. *citrinum* at Kiel (Pape, 1938).

Gäumann (1918, 1923) had previously recognized two subspecies of *Peronospora parasitica* (Fr.) Tul. within the host genus *Alyssum*, namely *Peronospora alyssi calycini* Gäum. on *Alyssum calycinum* and *Peronospora alyssi incani* Gäum. on *Alyssum incanum*. The conidia of *Peronospora alyssi calycini*, averaging 25 × 19 μ, are much larger than those of *P. galligena*, but according to Blumer the last-named species is not widely different morphologically from *P. alyssi incani*, or from *P. berteroae* Gäum on *Berteroa* (*Alyssum*) *incanum* and *Peronospora camelinae* Gäum. on *Camelina microcarpa*. Nevertheless, in inoculation experiments with *Peronospora galligena*, Blumer could infect only *Alyssum saxatile* and its varieties *citrinum* and *compactum*. He failed to infect twenty other crucifers, including *A. calycinum*, *A. incanum* and *Camelina microcarpa*, and therefore regarded his fungus as a distinct species. He also considered it to be distinct from a Downy Mildew found on the native *Alyssum saxatile* in Moravia and named by Hruby (1930), without description, *Peronospora alyssi arduini*. As a specialized form of the very common *P. parasitica* the fungus occurring on *Alyssum* in this country may well be widely distributed, though unnoticed. Over fifty years ago Halsted (1891) reported *Peronospora parasitica* in New Jersey on greenhouse plants of *Alyssum maritimum* growing near affected radishes.

35. Downy Mildew of the vine (*Plasmopara viticola*)

The Downy Mildew of the vine appears still to be quite rare in Great Britain, and some interest therefore attaches to new records of it. W. G. Smith (1886) was able to write of this mildew: 'at present, although expected every season, it has not yet been recorded from Britain', but within a few years M. C. C[ooke] (1894) reported that two sets of infected leaves had been received from places in Britain more than 100 miles apart—and from specimens in Herb. Kew and in Herb. Mus. Brit. it is certain that one of these places was Derby. During the next thirty years *Plasmopara viticola* (Berk. & Curt. ex de Bary) Berl. & de Toni was recorded a few times in different parts of the country, but little reliance can be placed on these records and one or two of them proved on closer inquiry to have been errors for *Uncinula necator* (Schw.) Burr., the true vine mildew. The third authentic record of the *Plasmopara* was not made until Harrison and Ware (1926) found it on an outdoor vine in a private garden at Wye, Kent. The infected vine was cut off at ground level at the time and the above-ground parts were burnt. The rootstock, which was left intact, grew vigorously during the next few years, and in September 1932 Downy Mildew was found in small quantity on the new growth. About the same time it was also collected on the ornamental *Vitis coignetiae* Pulliat in a garden at Haslemere, Surrey. The sixth known occurrence in Britain was in 1936 on a very old outdoor vine in a garden at Wye about 300 yards from the earlier infected vine, which had been pulled up and completely destroyed after the 1932 outbreak. In 1941 a substantial attack was observed on a Miller's Burgundy vine growing in the open at Wye on a house wall about a quarter of a mile from the earlier infected vines: though three plants, Brant, Chasselas rose (Reine Olga) and Early Saumur Frontignan, growing on the walls of the same house were not attacked. The eighth and most recent* record was made in August 1947, when Mr F. G. Ordish sent me mildewed leaves taken from a fairly severely affected Teinturier Grape, *Vitis vinifera* L. var. *purpurea*, growing in his garden at Yalding, Kent.

One of the first two outbreaks, and perhaps both of them, occurred under glass, for Cooke (1894) wrote that at one locality the vine attacked 'was a different variety from others which the house contained', but all the more recent records have been on outdoor plants. Early fears that *Plasmopara viticola*, once introduced, would rapidly become established in Britain have not been justified, and there is now no reason to suppose that it will ever become a menace here.

36. Pre-storage Black Heart in potato tubers

Black Heart is not uncommon in clamped potatoes and in tubers stored in badly ventilated places, and in the United States of America it has at times caused considerable loss among potatoes being transported in stove-heated railway wagons. Indeed, it is apt to occur whenever potato tubers are subjected to temperatures of 38–48° C. for at least fifteen to twenty hours, or at much lower temperatures if ventilation is poor and the supply of oxygen sufficiently low. Under such conditions the central part of the tuber is killed, and the dead part either becomes uniformly dark grey to purple or inky black in colour, or exhibits simple or intricate patterns on a somewhat lighter background. Sometimes the blackened tissues contract, giving rise to a smaller or larger hollow in the centre, with a black lining. In this state the defect may easily be confused with true Hollow Heart, a seasonal non-parasitic trouble observed chiefly in wet summers or when a drought in early summer is followed by rain in August or September. In Hollow Heart it is rare for the cavity to show a black lining.

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* A fairly severe attack occurred on a number of vines at Oxted, Surrey, in October 1948.

Observation has made it clear that Black Heart is not invariably a storage disease, but one that under special conditions may occur immediately after digging or even while the tubers are still in the ground. Temperature is a controlling factor, though there is obviously a close relation between the development of Black Heart, temperature, time of exposure, and oxygen supply. The first time I saw Black Heart in potatoes at digging time was during the late summer of 1933—a year that saw the hottest and driest August in England and Wales for at least thirty years. Specimens began to arrive in the first few days of September from gardens in the Bristol area, in Surrey and in other southern districts. In some tubers the whole of the central part was blackened, while in others there was only a small more or less central cavity surrounded by a brown or black lining. The same symptoms were also found to be fairly common in mid-Wales at lifting time, but it was not always clear, either there or elsewhere, whether the symptoms were present before lifting or whether they first developed a few days later, possibly after the potatoes had been left lying on the ground to dry in the sun. On one farm near Aberystwyth, however, the symptoms were known to have been present before digging. They were found in May Queen grown in light loam overlying shale, on a field with a southern slope of one in eight: while King Edward and Kerr's Pink growing alongside were unaffected. Other varieties affected that year were Sharpe's Express, King George and Up-to-Date. I did not see this pre-storage form of Black Heart again until 1938, when one sample only was received from Hailsham, Sussex. The variety was King George, grown in light soil: the affected tubers had been dug on 30 July, exposed to the sun over the weekend, and were found to have black centres on 2 August. Further complaints about this form of Black Heart were received in August 1947, during a month that for many parts of Britain was the sunniest and driest on record. From 19 to 28 August, specimens were received from Dorset, Sussex, Essex and the London area. Some of the tubers had been exposed to hot sun for two or three days after digging, but there was clear evidence in at least one instance that the tubers were affected 'as lifted'.

These observations strongly suggest that Black Heart may develop in potato tubers before or immediately after digging in seasons when temperatures in August are unduly high. As the defect cannot normally be detected until the tubers are cut, it is unwise to assume without more ado, when Black Heart is found during the winter months in clamped or stored tubers, either that the clamping conditions are unsatisfactory or that ventilation in the storage chamber is faulty. The true explanation may be that the symptoms were induced months before as an effect of high soil temperatures or of hot sun while the tubers were still attached to the parent plant.

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THE TEACHING OF MYCOLOGY

[A Report prepared by a Committee, appointed by the Council in April 1945, consisting of G. C. Ainsworth, Miss E. M. Blackwell, F. T. Brooks, C. G. C. Chesters, Miss L. Hawker, C. T. Ingold (*Chairman*), J. Ramsbottom, G. Smith, W. H. Wilkins and S. P. Wiltshire. The Report was circulated privately in June 1946.]

Foreword

The dearth of trained mycologists has been apparent for many years and had serious effects during the war period.

In 1944 the Council of the British Mycological Society issued a report on 'The need for encouraging the study of systematic mycology in England and Wales', drawn up by a specially appointed Committee. Constantly in its deliberations the problem of training arose, and, after some delay, the Council appointed a further Committee, composed mainly of those with practical experience, to report on 'The Teaching of Mycology'.

This Report was approved by the Council and it was decided to print and circulate it but to defer publication. J. Ramsbottom (*President*).

I. Mycology defined

Mycology is the study of all aspects of Fungi—their morphology, physiology, ecology, cytology, genetics, and taxonomy. Fungi form a huge group of probably some 100,000 species and their distinctive form and nutrition give to their study a special interest heightened by their enormous economic importance. Fungi are of great significance in industry and in medicine, but they are especially important as the principal causal organisms of plant disease. The great development of Plant Pathology during the past century has led some to the view that Mycology and Plant Pathology are synonymous, but this attitude is quite unjustifiable. Fungi should be studied primarily for their own sake, although, in a balanced course on Mycology, the economic aspects must receive their due emphasis.

II. Mycology in relation to botany

A good case can be made out for considering Fungi as a kingdom of organisms co-equal with Plants and Animals. However, the study of Fungi has developed, in the main, within the confines of Botany and on grounds of tradition, and also because of the paramount importance of Fungi in Plant Pathology, it is generally considered desirable that they should continue to be studied as part of Botany. Only a few of those who lecture on Fungi in the Universities are in favour of Mycology being taught as a separate subject like Bacteriology.

In an Honours course in Botany the amount of time given to the study of Fungi varies from one-tenth to one-sixth of the whole course. The Committee agrees that the course leading to a first degree in pure Science (as in Arts) should primarily be cultural rather than technical and that it, therefore, is not desirable in a Botany degree to emphasize one aspect of the subject at the expense of others. Nevertheless, they feel that Fungi, if properly treated, certainly deserve one-sixth of the available time.

It is suggested that for students taking an Honours degree in Chemistry, Mycology might form a more valuable subsidiary than Botany or Biology. If a course of this type were developed in any of the Universities, it would be most desirable that it should be a general course in Mycology and not a technical course in Industrial Mycology. The Committee regard it as a basic principle that the study of Fungi as a branch of pure science should always precede a study of Applied Mycology.

It should be noted that the teaching of Mycology in the Universities is not confined to the departments of Botany. Students studying for degrees in Agriculture, especially for Honours degrees in Agricultural Botany, follow special courses usually conducted by lecturers of the Faculty of Agriculture. Again in at least one Department of Biochemistry, a School of Industrial Mycology is developing. The fact that Mycology is no longer the monopoly of Botany should have a stimulating effect on the development of the subject.

Most departments of Botany have a lecturer on the staff with special qualifications in Mycology. It is only in the smaller departments that this is not so. This involves no criticism of these small departments, which obviously cannot have a specialist in all the important branches of Botany.

Many departments have special mycological laboratories, but others with cramped quarters have not, and cannot expect to have such facilities. However, it is essential that all Botany departments should be fully equipped for the special discipline of mycological work, including adequate library facilities. Liberal supplies of apparatus are necessary if all post-intermediate students are to have the opportunity of studying Fungi in the laboratory as living organisms and of carrying out experiments in pure culture.

III. THE CONTENT OF MYCOLOGY

Fungi have suffered in the past from their relegation to a subphylum of the Thallophyta with the implication that they are degenerate Algae. Like Algae, Fungi have tended to be studied from the standpoint of evolutionary morphology with over-much emphasis on sexuality and cytology. The 'type system' has largely dominated their treatment at the intermediate and subsidiary levels.

The Committee suggests that in the study of Fungi their consideration as living organisms is of special importance. Botany is primarily an observational science, but an increasing element of experimental work is desirable. The technique of isolating and growing Fungi in pure culture, and the study of their physiological reactions under these conditions is simple and interesting, and is not only of great value from the standpoint of Mycology, but also from the point of view of the experimental method in Botany. However, it is undesirable that Fungi should be treated merely as units in a collection of type cultures. Where possible, some species studied by the student should be isolated by him from natural substrata.

A study of Fungi as living organisms involves not only an extension of culture work, but also an extension of field work. Individual practical work by Honours students on particular systematic or ecological groups of Fungi is of special value in promoting the spirit of modern Mycology. An Honours student might well undertake a special study during his course. He might study the bark-inhabiting Fungi of a tree, or the rusts and smuts occurring in his neighbourhood, or investigate the succession of species which develops on horse dung, or study the distribution of Fungi in a wood, or make observations on the watermoulds of a stream, and so on. A student, in this way, by first-hand contact with Fungi, will gain an insight into Mycology which can be obtained in no other way. He will not only learn to know Fungi in nature, but will also become acquainted with some mycological literature and with its use in the identification of genera and species.

Although in a B.Sc. course Fungi should be studied primarily for their own sake, the fundamental facts of their importance, both beneficial and destructive, in Agriculture, Forestry, Industry, and Medicine, should receive more emphasis.

Bacteriology has evolved into a separate science, but bacteria, because of their fundamental physiological similarity, should find a place in a course on Fungi. Their practical study should be more than a mere examination of a few types under the microscope and should include: (1) the method of identification based on physiological tests; (2) the estimation of bacterial numbers (e.g. in soil); and (3) the chief staining methods.

IV. Post-graduate work

The Committee has no comment to make on the conduct of mycological research. The development of this in the Universities should continue to be free and unfettered.

It may be noted, however, that the volume of mycological research in this country is small and, proportionately, compares unfavourably with that in America. Last century France and Germany led the world in the study of Fungi. The lead has now passed to America. If this country is to play its proper part in the future development of Mycology, much larger sums must be available for research, both fundamental and applied.

The Committee suggests that it is desirable to develop post-graduate courses in Mycology. Although in certain University departments Mycology in the Honours degree receives very considerable emphasis, the Honours graduate in Botany cannot normally be regarded as one sufficiently trained in Mycology to be able at once to fill a mycological post or to engage in mycological research.

Certain post-graduate courses already exist, but there does seem to be a real need to develop further advanced teaching in Mycology as distinct from Plant Pathology, and it is suggested that, in those Universities where the M.Sc. degree may be awarded on examination, an M.Sc. (Mycology) would be a most desirable degree to institute.

In a post-graduate teaching course in Mycology the discipline of the subject and all its aspects should be treated without too much emphasis on any one of them. Such a course would, it is believed, be very suitable for a student who subsequently or concurrently is engaged in the study of Plant Pathology.

It is clear that not all University centres will wish to develop post-graduate courses in Mycology, but it is desirable that a number of such centres of higher mycological teaching should exist.

If a number of post-graduate teaching courses are instituted in the Universities, the character of each will naturally depend on the special branch of Mycology in which the head of the department is most interested. It is, therefore, necessary to have a scheme of co-operation amongst the Universities so that a post-graduate student may be able to select the department in which he wishes to study in accordance with his own special interests, and so that a suitable graduate from a University, not offering a post-graduate training in Mycology, may freely pass to a centre offering such training and be eligible for the degree or diploma at the end of the course.

THE NEED FOR ENCOURAGING THE STUDY OF SYSTEMATIC MYCOLOGY IN ENGLAND AND WALES

[A Report prepared by a Committee, appointed by the Council in October, 1942, consisting of G. C. Ainsworth, Miss E. M. Blackwell, K. St G. Cartwright, R. V. Harris, W. C. Moore (*Chairman*), J. Ramsbottom, G. Smith, and Miss E. M. Wakefield (*Convener*). The Report was circulated privately in July 1944.]

FOREWORD

A critical position in the study of the systematics of fungi in this country has been reached due to the shortage of experienced taxonomists and the absence of facilities for training in taxonomy and nomenclature. The urgency of this problem being apparent to the British Mycological Society, the Council appointed a Committee to study the question and draw up a Report. The Report presented was warmly approved by the Council who decided to circulate it forthwith to all Government Departments, Universities, Institutions and Associations concerned with the activities of fungi. It was decided that the Report should be printed, for convenience, but that it should not be published then, either as a whole or in part. R. W. MARSH (*President*).

INTRODUCTION

The study of the systematics of fungi has been seriously neglected in this country. During the past thirty years increasing emphasis has been placed on the economic aspects of plant pathology, but there has been a lack of appreciation of the importance of taxonomic mycology to the plant pathologist. Yet increasing importance has been attributed to the damage caused by fungi not only in agriculture and forestry, but to man and animals, in textile industries and in stored products. Their significance in soil fertility, in fermentation industries, and in the production of therapeutic substances is gaining wider and wider recognition. The degree courses in botany at the universities, as they are at present arranged, do not permit adequate training in the taxonomy and nomenclature of fungi. This, added to the fact that the number of experienced taxonomists is diminishing, and few, if any, are being trained to replace them, makes the position critical.

The following Report has been prepared to draw attention to the urgent need for action. In it the history of mycology in this country is traced and a brief account is given of the present position of systematic mycology both at home and abroad. This is followed by a reasoned statement of the necessity for stimulating the study of the subject at the universities, in the central herbaria and in laboratories dealing with economic mycological problems. Certain specific recommendations are made.

HISTORY AND PRESENT POSITION OF MYCOLOGY IN ENGLAND AND WALES

The comprehensive study of mycology in this country may be considered to have begun with Miles Joseph Berkeley, a clergyman who took up the study of fungi as a hobby. He published his first mycological work in 1836, and from then until his death in 1889 he was responsible for numerous papers and books dealing with fungi collected in all parts of the world. Besides adding greatly to our knowledge of species and genera of fungi and of their distribution, Berkeley recognized that fungi are the cause of decay and disease and so laid the foundations of the science of plant pathology in this country. Following him M. C. Cooke and G. Massee began as amateurs, but eventually obtained official positions at Kew. Worthington G. Smith was a botanical artist who became interested in mycology and worked at the British Museum; C. B. Plowright, who became an authority on the rust and smut fungi, was a medical man. Thus, most of the important early work in mycology in this country was done by men who took it up in their spare time, and distinguished 'amateurs' have continued this valuable work to the present day, though in decreasing numbers. For very many years the terms mycology and plant pathology were regarded as practically synonymous, and such paid posts as existed arose from the need of a 'curator' for national collections, or from a demand for advisory or research work on plant diseases. The only Government posts for mycologists were originally at the British Museum and Kew.

British Museum. In 1859 W. Carruthers was appointed Assistant in the Department of Botany, then at Bloomsbury, and in 1871 he became Keeper. He worked mainly with cryptogams and fossil plants but when, in 1871, he also took over the post of Adviser in Botany to the Royal Agricultural Society he was compelled to study plant diseases—work in which he was later assisted by Miss A. Lorrain Smith. G. R. M. Murray, an Assistant in the Department, had charge of fungi and algae from 1876. When he became Keeper in 1895, V. H. Blackman was appointed Assistant in charge of fungi, and was followed (1907–9) by W. E. St J. Brooks. In 1893 Miss Lorrain Smith was employed by the Trustees in arranging the stands illustrating microfungi in the Botanical Gallery and she also took over practically all the identifications of microfungi. By 1910, however, her time had become fully occupied with lichens. J. Ramsbottom (Keeper of Botany since 1930) was appointed Assistant in charge of fungi in 1910. In 1929 Miss F. L. Stephens was engaged to assist in laboratory and routine work, and she was given a permanent appointment in 1936 with the title of Scientific Assistant. Thus, the mycological work in the Department is now carried out by the Keeper (who is much occupied with heavy administrative duties) and a whole-time Scientific Assistant. Since 1930 there have been additional appointments in the Cryptogamic Herbarium, but the full scheme of development with the consequent increase in the number of mycologists has not yet been implemented.

Royal Botanic Gardens, Kew. In 1880 M. C. Cooke was appointed to take charge of the lower cryptogams in the Herbarium at Kew, and between 1880 and 1892 he completely rearranged the fungi there and incorporated many large collections, including his own, while at the same time he published many papers and books on fungi. G. Massee worked with him for some years in a voluntary capacity, and when Cooke retired succeeded him in charge of the lower cryptogams in 1893. From then, until his retirement in 1915, Massee was responsible for both systematic work on fungi and advisory work on plant diseases, not only for the Ministry of Agriculture but also for the Empire. In 1904 A. D. Cotton was appointed as a second Assistant in the Cryptogamic Department, and worked especially with lichens and algae, though he also carried out important mycological investigations. In 1910 Miss E. M. Wakefield was appointed to assist Massee. In 1915, not long after Massee's retirement, Cotton was transferred to the post of Mycologist (Plant Pathologist) in the recently founded Plant Pathology Laboratory of the Ministry of Agriculture and the Kew Herbarium was thus once more reduced to one Assistant in charge of all the lower cryptogams. After the first world war some intermittent help in mycology was obtained from temporary technical assistants, who required much of the Assistant's time to train them and who usually left for more remunerative posts as soon as they had become useful. At present there is still only one mycologist on the staff.

Imperial Mycological Institute. In 1921 a further development took place when the Imperial Bureau of Mycology (now the Imperial Institute) was established under the direction of Dr E. J. Butler to deal primarily with problems of mycology and plant diseases in the overseas parts of the Empire. Here at least the increasing need for systematic work on fungi has been acted upon, but of the five mycologists on the staff only two are sufficiently free from routine and advisory work to be able to devote time to any special systematic problems which arise.

In addition to those referred to above there are a few academic and amateur mycologists who have specialized in certain groups of fungi.

THE POSITION OF SYSTEMATIC MYCOLOGY ABROAD

It is relevant at this stage to refer briefly to the position of systematic mycology in other countries.

Europe. Generally speaking, the position throughout Europe has been much the same as in this country as regards professional posts in systematic mycology. Nevertheless, considerable research work in the subject has been carried out on the Continent by individual pharmacists, school teachers and others who have had training in botany or have become interested in mycology, and by plant pathologists and members of university staffs who work at systematic problems in their spare time. Special mention may be made of Holland, for there a flourishing mycological school has grown up in connexion with the University of Utrecht and the Willie Commelin Scholten Laboratory and Central Bureau for Fungus Cultures at Baarn. It is therefore not without significance that mycologists trained in Holland have done much valuable work in the Dutch colonies, notably Java. Reference may be made also to the Carlsberg Laboratory at Copenhagen, which has been active in the taxonomic study of alcoholic fermentation organisms.

United States of America. On the other side of the Atlantic the position is very different. Mycology is taught at most universities, and because posts in systematic mycology are available, post-graduate students take up the study of special genera or groups of species, with the result that valuable work is being published. Many Agricultural Experiment Stations and State Agricultural Colleges have mycologists on their staffs who carry out research on special groups of fungi, as, for instance, the work on rusts at Purdue University Agricultural Experiment Station. The United States Department of Agriculture, in connexion with the Bureau of Plant Industry, maintains large mycological collections, with one Senior Mycologist in charge and three Assistant Mycologists. There are also large herbaria at various universities and other institutions. In fact great and active interest is widely taken in fundamental problems relating to the taxonomy and nomenclature of fungi, as evidence of which it may be mentioned that the important American Phytopathological Society recently appointed special committees, one for the consideration of fungus nomenclature and the other for the publication of monographs.

British Empire. Valuable critical work on fungi is now being carried out in Australia, New Zealand, Canada, India and South Africa. Particular mention might be made of the very active work on fungi in the Union of South Africa, which possesses a good National Herbarium from which a number of excellent monographs have been published. Of the Colonies and Dependencies overseas, Ceylon is the one where fungi have been collected and studied most widely. The early collectors laid the foundations of a good herbarium at Peradeniya, and subsequently the work of a former Government Mycologist very greatly extended our knowledge of the Ceylon fungus flora. Similar work has been begun more recently in other Dependencies, notably Uganda, Sierra Leone, Singapore and Bermuda. Keenly interested Government Mycologists began by sending large collections to this country to be named at Kew, the British Museum and, in later years, at the Imperial Mycological Institute. As a result of this helpful and essential collaboration the overseas mycologists have gained experience which has enabled them to build up their own herbaria and carry out independent monographic work of fundamental importance.

THE NEED FOR ENCOURAGING SYSTEMATIC MYCOLOGY

Fungi are among the commonest agents of disease in plants. Thus, in the 1941 *Review of Applied Mycology*, in which the world's literature dealing with diseases of plants (except those caused by animal parasites) is surveyed, over 70 % of the papers on plant diseases abstracted dealt with fungus pathogens.

As has already been pointed out, the study of plant pathology has been greatly developed and encouraged during the past thirty years, and systematic mycology plays a vital part in this subject. It is true that with the development of the Ministry of Agriculture's Advisory Service after the last war, certain posts for 'Advisory Mycologists' were established, but these officers are primarily concerned with plant pathology and not all of them have had the opportunity of making studies in pure mycology. There are now about fifty persons holding posts outside the teaching staffs of universities, who are engaged in work on those aspects of plant pathology concerned with fungi.

Unfortunately, during the same thirty years, there has been little or no official recognition of the importance of the study of fungi as fungi, and the fifty plant pathologists now at work are still largely dependent for advice on and help with their problems in systematic mycology on three already overworked official taxonomists (one at Kew and two at the British Museum), two of whom are approaching the retiring age. Assistance, though necessarily limited, has been given, especially in recent years, by the Imperial Mycological Institute. Nevertheless, as outlined below, the position is very serious and needs immediate attention.

University training. The amount of instruction in systematic botany included in the degree courses varies considerably; in English universities it is generally very meagre. Although lectures on fungi and their life cycles are given, there is little instruction in the taxonomy and nomenclature of fungi. The amount of encouragement given to taxonomy depends almost entirely on the interests of the Professor and candidates for higher degrees are rarely, if ever, advised to take up taxonomic problems. One reason for this is undoubtedly that there are so few posts open to taxonomists. It is doubtful if any university provides a course on nomenclature, and the technique of examination of herbarium specimens is generally unknown to students entering applied mycological posts. Courses in general mycology in this country do not compare unfavourably, on the whole, with those, for instance, in America, and a number of Professorships or Lectureships in Botany in our Universities and University Colleges are held by individuals keenly interested in mycology, but these courses do not include adequate training in taxonomy. In our view systematics is an essential part of any problem in phytopathological, industrial or medical mycology. One need is for applied mycologists with sufficient knowledge of the fungi and of the literature concerning them to be capable of identifying the common species which are met with as pathogens, or in industry, or as sources of human and animal food. Such workers would relieve mycologists at the central Herbaria of much routine work, enabling them to turn their attention to much needed revision and research work. If the posts are provided, a demand will be created for post-graduate systematic training in the Universities. This may have to be met in the first instance through the help of the few systematists now holding official appointments. Facilities for post-graduate training in systematic mycology are essential if the present state of affairs is to be improved. It is a sad commentary on the training of plant pathologists in systematics that none of the agricultural or horticultural research stations in this country maintains a working mycological herbarium.

Central herbaria. It cannot be too strongly emphasized that research work on a parasitic organism has little permanent value until the organism has been correctly identified and its relationships understood. Failing this, unnecessary confusion and duplication of work may result and the accumulated knowledge of the pathogen under various names will not be fully available. Lack of good advice on systematic mycology, or inadequate knowledge, has undoubtedly led to the literature becoming encumbered with a vast number of names because authors have not been familiar with or have not appreciated previous work. The applied biological sciences, such as agriculture, horticulture, forestry, medicine, and many industrial undertakings which either use or are affected by fungi, are dependent on qualified taxonomists to whom mycological problems can be referred, as the requisite specimens and literature necessary are centred in the larger herbaria. Moreover, the day is long past when the whole field of the fungi can be covered by one individual. While plant pathology has been developing on the one hand, systematic mycology has also been increasing in extent and complexity. Not only have numerous new genera and species of fungi been recognized in the past few decades, but the increased use of the microscope and of cultural methods by the pathologist have helped to revolutionize taxonomic work. New ideas of the value of both morphological and biological characters are coming into being, which may have far-reaching effects on classification and nomenclature. Careful revisions of certain genera and families of fungi in the light of modern knowledge are urgently required and as fresh knowledge accumulates every group needs repeated revision. To meet all these demands adequate central facilities are necessary, and these can be provided only at the great National Herbaria at the Royal Botanic Gardens, Kew, and the British Museum (Natural History), at the Imperial Mycological Institute and at certain of our Universities. At all these places it should be possible for fundamental research on taxonomic problems to be carried out unfettered by demands for results of immediate economic importance. Such work requires long training, which cannot be included in any degree course. It is no exaggeration to say that ten years' work in a herbarium is necessary before a mycologist begins to acquire the general knowledge and sound judgement which is essential to a good taxonomist.

As already explained, Kew has one mycologist, and the British Museum two, one of whom is also Keeper of Botany. These officers are responsible for the upkeep of collections, for routine correspondence and extensive advisory work, and for assisting workers at home and abroad by the investigations of type specimens, etc. in the collections under their care. Consequently there is little or no time for research, and in this respect England is falling sadly behind in mycological work as compared with North America. The Imperial Mycological Institute has two mycologists working on taxonomic problems, but by the terms of reference of the Institute their work must usually have some bearing on plant pathology, and especially the problems of the Empire overseas. The number of experienced workers is thus dangerously small. Apart altogether from the question of carrying out original work in mycology, it is highly probable that unless steps are taken in the near future to rectify matters, not only will it become increasingly difficult to train students, but it may soon become impossible for the applied mycologist to obtain an authoritative identification in this country of organisms belonging to certain groups of fungi.

Research Stations and advisory service. The liaison between the Research Stations and systematic mycology is twofold. When a fungus pathogen, proved or suspected, has been isolated by a pathologist and he is uncertain of its identity, the present procedure is to submit specimens or cultures to taxonomists at the central Herbaria. The systematic specialists at these institutions are better equipped both by experience and reference facilities for much of this work, and but for their co-operation much valuable time and accuracy would inevitably be sacrificed by the pathologist. The examination of such material, however, places an additional routine burden on the present very restricted number of pure systematists, and the rapid expansion of activities in pathology at the Research Stations clearly calls for some expansion in the staffs at the central Herbaria. Nevertheless, at least some of this work of identification could and should be done at the Research Station itself.

Concurrently the pathologist carries out experiments on the biology and pathogenicity of his fungus, during the course of which a body of valuable and essential data on the effect of host and other environmental conditions is obtained, and this is of the utmost reciprocal value to the systematist for determining new standards in classification and nomenclature. To carry these studies to their final stages an appropriate taxonomist from the central Herbaria might profitably be temporarily attached to the Research Station Pathology staff, in order to make the necessary 'field contact' with the fungi in question. Present experience at Research Stations shows that the pathologist needs a sound grounding in fungus systematics for the efficient elucidation and application of these biological data. Pathologists lacking this background inevitably tend to regard the morphological identity of the pathogenic fungi as of secondary or even negligible importance. To counter this it is felt that an adequate course in systematic mycology should form part of the training of every Research Station pathologist working on fungus diseases, and that full facilities for specializing in taxonomy should be given to those who show leanings in this direction. Such specialization would normally include some months' training at one of the central Herbaria.

The same considerations apply in the liaison between systematic mycology and the provincial Advisory Mycologists. Close collaboration between the central Herbaria and Advisory Mycologists is very necessary, but in addition the Adviser must look to his headquarters at Harpenden for assistance with many of his mycological problems, and to meet this need mycology (as distinct from plant pathology) needs strengthening at the Ministry of Agriculture's Plant Pathology Laboratory at Harpenden.

Industrial mycology. All industrial products and raw materials of an organic nature are liable to attack by fungi and almost all manufacturers and merchants of such products experience trouble from mildew or mould at some time. In comparatively recent years much has been done in some industries to develop methods of control, particularly in the cotton industry, in leather and rubber manufacture and in the food canning industries. A very serious hindrance to rapid progress in industrial mycology, however, is the difficulty of identification of the organisms responsible for damage to products. Brewers, distillers, cider manufacturers and wine producers are dependent upon definite strains of yeast and not only must these be maintained free from contamination by wild yeasts of rather close affinity, but also from non-sporulating forms which cause disease. In addition there is increasing use of moulds in fermentation industries. Citric acid is now produced almost exclusively by mould fermentation, and gluconic acid, lactic acid and tannic acid can be manufactured in similar ways. Fats, glycerol, enzymes, alcohol, vitamins and other desirable products can all be obtained on a large scale by fermentation processes. A recent application is the manufacture of 'food yeast', rich in protein and the vitamin B complex, by growing a non-sporing yeast on molasses. Anti-bacterial and anti-fungal substances, such as penicillin, patulin and gliotoxin, are the products of definite species of moulds. In all these useful applications of microfungi it is essential that the organisms be correctly identified. At present identifications are often carried out by one worker for another as a matter of courtesy. This usually means that an expert in a particular group of fungi, already fully occupied with his own investigations, is overwhelmed with requests for assistance. It also means that the inexperienced worker, who particularly needs assistance, may, from lack of the necessary personal contacts, be unable to obtain the help that is needed. The difficulties could be largely overcome by extending, on the industrial side, the scope of the National Collection of Type Cultures, and it is recommended that a specialist in industrial fungi be appointed to its staff.

Forest and timber pathology. Systematic study is essential for the solution of many outstanding problems in forest pathology. Correct naming of organisms responsible for leaf and seedling diseases is difficult, and there is a great deal of confusion even about the larger fungi that cause Heart Rot. Identical fungi in America and Europe have been given different specific names. In timber pathology a similar state of affairs exists and reference often has to be made to American and Continental authorities. In practice a wrong identification may lead to serious consequences, because intensive work carried out on one organism may lead to the adoption of control measures which if applied against a different pathogen may prove useless.

A collection of cultures can be used as standards only if the original isolations have been made from correctly named sporophores. Otherwise great confusion may result. The preparation and maintenance of such a collection should always be in the hands of specialists in the different groups.

Medical mycology. The whole subject of medical mycology in this country calls for consideration. There appears to be no course of lectures at any Medical School except a short one for overseas medical men at the London School of Hygiene and Tropical Medicine. So far as taxonomy is concerned the accounts of fungi published in British medical works give clear evidence that some fundamental training is necessary. Compared with what has been done in France, and what is now being achieved in the United States, the amount of sound work accomplished in this country is insignificant. It is understood that the Medical Research Council has recently appointed a Committee to report on the matter and that taxonomy and nomenclature are being considered as a special aspect of the problem. Comment is therefore restricted to the observation that the existence of the National Collection of Type Cultures is of the utmost importance and to emphasizing the need for a medical mycologist on the staff. In obtaining cultures from various investigators it acts as a check on haphazard naming and can provide material for investigation and for comparative studies.

RECOMMENDATIONS

It is apparent that the difficulties facing systematic mycology in this country cannot be overcome rapidly. There is a great demand for mycologists qualified to undertake taxonomic work. Fungi are not only agents of disease or spoilage: they are also used in many industrial processes, in medicine, and to some extent for food. There is need for mycologists who can identify correctly the species and strains of fungi concerned, who can collect the morphological and biological data essential to the systematist for his taxonomic studies, and who can carry out monographic studies of fungus genera and families in the light of modern knowledge. To meet this need it is recommended that:

(1) The staffs for systematic work at the two National Herbaria, the Royal Botanic Gardens, Kew, and the British Museum (Natural History), and at the Imperial Mycological Institute should be increased sufficiently to ensure a continuity of expert advice and to allow adequate opportunities for research.

(2) Facilities should be provided for the temporary attachment of taxonomists from the central Herbaria to the staffs of institutions dealing with applied mycology when field contact with a particular group of fungi is essential for further taxonomic study. Opportunities should also be afforded for research and advisory pathologists to be seconded to work in the central Herbaria for short periods.

(3) An effort should be made to ensure that plant pathologists are given more intensive training in mycological taxonomy and that they are afforded facilities for specialization in this subject.

(4) At the Plant Pathology Laboratory, Harpenden, mycology should be given increased attention. For this purpose the appointment of a taxonomic mycologist is suggested.

(5) A specialist in industrial fungi should be appointed to the staff of the National Collection of Type Cultures. This would enable arrangements to be made for private firms and research associations to obtain identifications of moulds. Later, the service might be extended to giving advice on industrial problems.

(6) It is suggested also that a medical mycologist should be added to the staff of the National Collection of Type Cultures.

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